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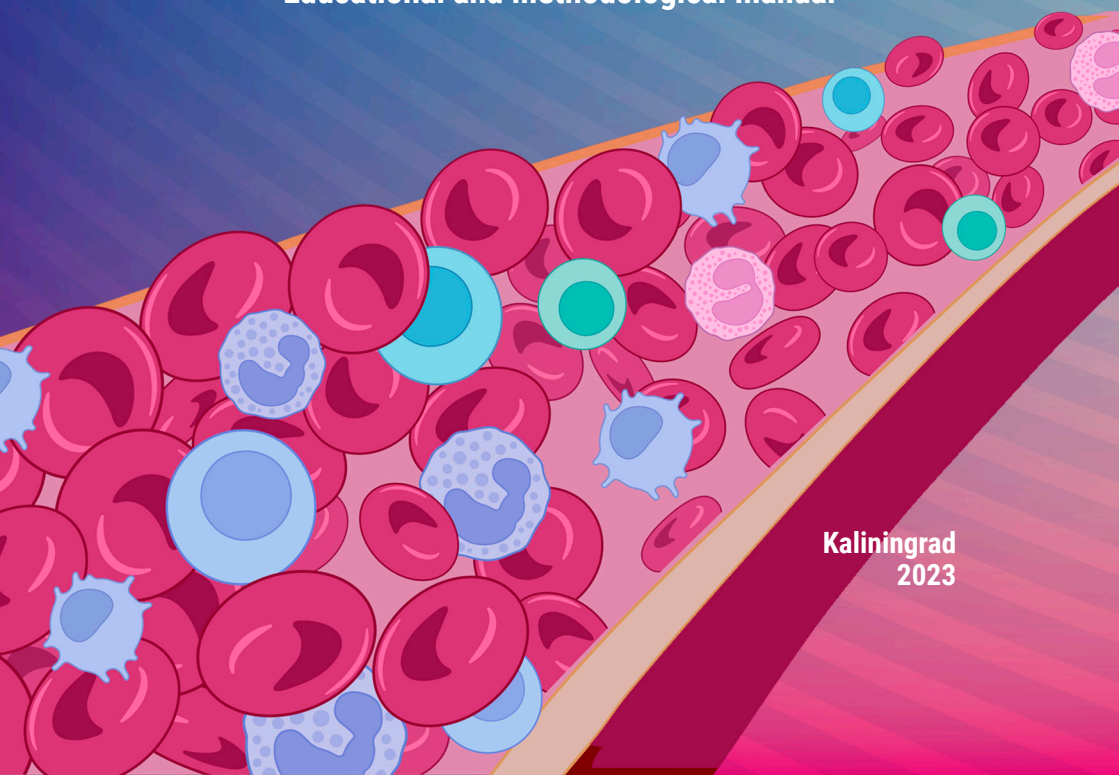
PATHOPHYSIOLOGY OF BLOOD

Educational and methodological manual



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Immanuel Kant Baltic Federal University Press
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The educational and methodological manual illuminates and analyzes the pathophysiological mechanisms of the development of disorders in the blood system. The manual presents clinical and laboratory signs as well as an algorithm for the diagnosis of anemia, leukocytosis and leukopenia. Much attention is paid to the etiology and pathogenesis of hemorrhagic diathesis and thrombophilia. In the description of the algorithm for the diagnosis of pathologies of the hemostatic system, the approaches developed in our country were used. The educational and methodological manual was developed in accordance with the work program of the specialty “Pathological Physiology” and is intended for students of medical universities and students of advanced classification faculties.

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CONTENT

TOPIC 1

PATHOPHYSIOLOGY OF RED BLOOD

Purpose of the session:

To learn modern ideas about hematopoiesis, etiology, pathogenesis, clinical and laboratory manifestations, the principles of anemia diagnosis, to study the pathological forms of red blood cells and the peculiarities of the morphological composition of peripheral blood in various types of anemia, to define the concept of erythrocytosis, to study the causes and pathogenesis of the development of erythrocytoses.

It is necessary to:

— have an understanding of the pathological processes that may occur in patients with morphological and functional disorders in organs participating in the processes of hematopoiesis and blood destruction, as well as the mechanisms of dysregulation of erythropoiesis;

— know the etiology, pathogenesis, mechanisms of clinical manifestations and principles of treatment and prevention of anemia and erythrocytosis;

— be able to solve typical situational tasks and test tasks on the topic of the lesson;

— have the skill of making a diagnosis based on the peculiarities of the morphological picture of peripheral blood in various types of anemia and the ability to give a hematological characteristic of various types of anemia, using morphological criteria based on their classifications.

Anemia is a condition characterized by a decrease in hemoglobin concentration and, in most cases, the number of red blood cells and hematocrit per unit blood volume.

The criteria recommended by WHO experts for the diagnosis of anemia in women are hemoglobin (Hb) concentration less than

120 g/L, red blood cell (RBC) number less than 3.8 million/μl, hematocrit (Ht) less than 36%, in men Hb<130 g/L, RBC<4.0 million/μl, Ht<39% (Table 1).

Table 1

Parameters for the study of red blood cells

Decryption	Parameter	Explanation to Parameter
NRBC	Normoblasts	Nucleated red blood cells
@LHD	Calculated indicator, reciprocal	% of red blood cells with reduced content Hemoglobin — hypochromic erythrocytes; reference values: <5.7%, with hypochromia — increases
@MAF	Calculated indicator, dimensionless quantity	Microcytic anemia ratio @MAF=(HGB×MCV)/100, an indicator of latent iron deficiency. RV: 10.6—15.5 HGB hemoglobin
@RSF	Calculated indicator	RSF=$\sqrt{(MRV \times MCV)}$ Characterizes the size of the red blood cell, the indicator latent iron deficiency. RV: 85.7—100.8 fl
HGB	Hemoglobin level	
RBC	Red blood cell count	
HCT	Hematocrit	
MCH	Average hemoglobin content in an erythrocyte	
MCV	Average red blood cell volume	
MRV	Average reticulocyte volume	
MCHC	Average hemoglobin concentration in erythrocyte	

Anemias — are diverse in their genesis and often have a mixed pathogenesis. In most cases, anemia is not an independent nosological form, but a manifestation of the underlying disease (Fig. 1, Fig. 3, Fig. 4).

Incidence of different types of anemia: color;

• 37% Iron deficiency anemia (FeDA);

• 27% Anemia in chronic diseases (ACH);

• 12% Anemia caused by erythropoiesis disorders in infections, megaloblastic anemia, refractory anemia, thalassemia;

• 10% Hemolytic anemia;

• 9% Anemia in renal failure and endocrine disorders;

• 5% Anemia in aplasia.

LHD% — Low Hemoglobin Density %;

MCV — mean volume of one red blood cell % red blood cells with reduced hemoglobin content — an indicator of hypochromia. Used to investigate the status of iron in the patient's body;

@ MAF factor is required for differential diagnosis of latent iron deficiency anemia from other forms of anemia (e.g., chronic disease anemia). So, 15% of women suffer from iron deficiency anemia with hemoglobin values within the normal range. This phenomenon is called latent iron deficiency anemia.

LID (Latent iron deficiency)=Hb (N) and Ferritin (↓) The clinical significance lies in the early diagnosis of latent iron deficiency anemia, as well as to clearly differentiate it from the anemia of chronic conditions.

@RSF, a factor of erythrocyte size, characterizes the size of the erythrocyte throughout life, including mature erythrocytes and their young forms (normoblasts and reticulocytes). Since the availability of iron can influence the size of red blood cells, this parameter makes it possible to judge the effective supply of iron in the body for erythropoiesis (Fig. 2).

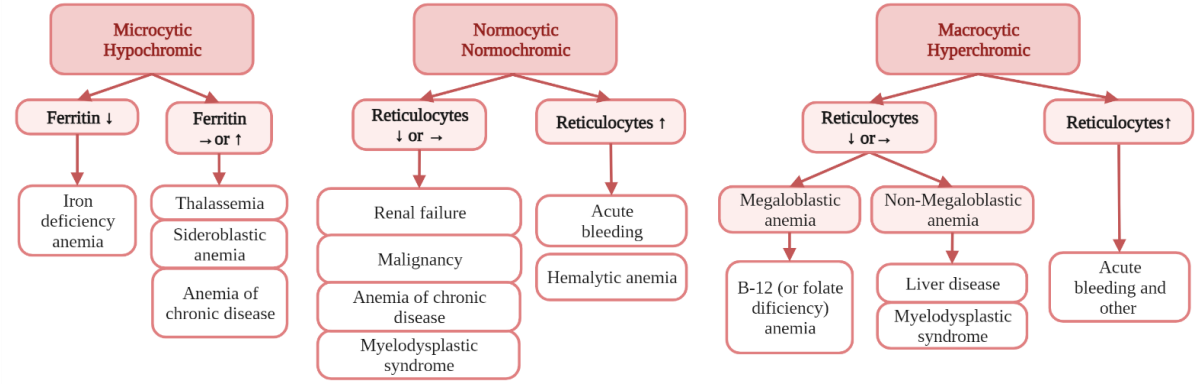

Fig. 1. Diagnosis of anemia

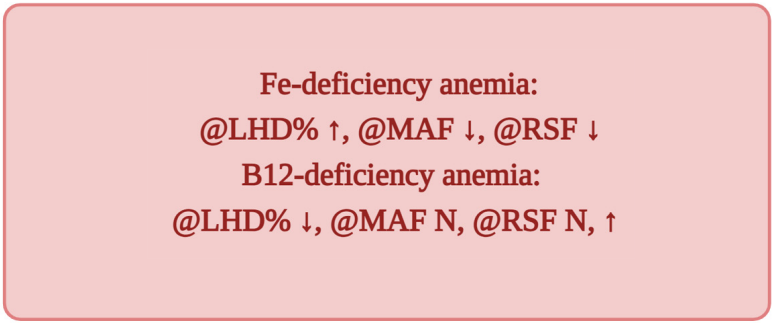

Fig. 2. Iron deficiency anemia and B-12 deficiency anemia

Biochemical parameters for the diagnosis of anemia

Serum iron (Fe).
Unsaturated Iron-Binding Capacity (UIBC).
Total Iron-Binding Capacity (TIBC).
Transferrin.
Transferrin saturation.
Ferritin.
C-Reactive protein.

Serum iron (Fe).

Reference values in adults, i.e., over 14 years old, are in the range: for women — from 9 to 30 μmol/l, for men — from 12 to 31 μmol/l.

***Diagnostic significance*:** iron ↑ in hemolytic, pernicious and aplastic anemia.

Transferrin (TF).

Normally, the level of transferrin is from 2 to 3.6 g/l, while in women it is 10% higher, and in pregnancy, especially in the last trimester, it can increase by 50%. Another reason for the increase in blood transferrin, usually combined with a drop in iron levels, may be iron deficiency anemia.

Function:

• the main plasma protein is an iron transporter;

• main iron donor for hemoglobin production;

• 1 mg of TF binds 1.25 μg of iron;

• Under physiological conditions, TF is saturated with iron by about 30%;

After iron release from the TF complex, the Fe^{3+} ion should be reduced to Fe^{2+}.

Acute inflammation contributes to a decrease in TF levels. «Negative acute phase protein».

Clinical relevance: the main clinical indicator for differential diagnosis between iron deficiency (TF↑) and hemolytic anemia (TF↓).

A more accurate indicator than total iron binding capacity of serum (TIBC).

Ferritin.

Reference values are shown in table 2.

Table 2

Reference values for ferritin

Age	Reference values, ug/L
1—2 months	200—600
2—5 months в	50—200
5 months — 15 years	7—140
Male over 15 years of age	20—250
Female over 15 years of age	10—120

Localization: ferritin accumulates in the liver, spleen, muscle and bone marrow.

Function:

• Form of iron deposition in the body;

• Ferritin-bound iron is used for heme synthesis (in intensive hemoglobin synthesis);

• Incorporation of iron into ferritin requires pre-oxidation of Fe^{2+} to Fe^{3+}.

Clinical relevance: the most specific and sensitive parameter of the presence of iron reserves in the body.

Immunochemical parameters for the diagnosis of anemia.
Serum folate.
Red blood cell folate.
Vitamin B-12.
Erythropoietin.
Antibodies to the internal factor of Castle.
Soluble transferrin receptor (sTfR).

Trasferrin receptor (TfR) and soluble transferrin receptor (sTfR).

TF receptor is a transmembrane protein with a molecular weight of 95 kDa. Soluble TF receptor (sTfR) is a shortened form of the TF receptor. Stable polypeptide 100 AA long. 80% of TF receptors are on erythroid cells.

Soluble transferrin receptor (sTfR): biological activity

• The number of TF receptors on the surface of cells depends on the concentration of iron in the cytoplasm, that is, on the cell's iron needs;

• The amount of soluble serum TF receptor is proportional to the amount of TF receptor on the cell surface and reflects the rate of erythroid cell renewal (erythropoiesis intensity);

• Assessment of sTfR-F index: [sTfR]/Log[Ferritin] is relevant.

With a lack of iron, the amount of soluble TF receptor in serum increases.

Tests needed to diagnose anemia

1. Study of all blood cell parameters in the complete blood count (CBC).
2. Serum iron (clinical biochemistry).
3. UIBC (clinical biochemistry).
4. TIBC (clinical biochemistry).
5. Transferrin (clinical biochemistry).
6. Transferrin saturation (clinical biochemistry).
7. Ferritin (clinical biochemistry).
8. CRP (clinical biochemistry).

9. Serum folate (immunochemistry).
10. Red blood cell folate (immunochemistry).
11. Vitamin B12 (immunochemistry).
12. Erythropoietin (immunochemistry).
13. Antibodies to the internal factor of Castle (immunochemistry).
14. Soluble transferrin receptor, sTfR-F index (immunochemistry).

Stages of anemia diagnosis:

1. Single-step automatic blood cell testing of all blood cell parameters as part of a screening CBC.
2. Select additional patient examination parameters based on CBC data.
3. Performance of biochemical and immunochemical tests justifying the final diagnosis.
4. Dynamic follow-up according to a number of necessary parameters, taking into account the diagnosis and therapy.

Figures 3—5 show examples of anemia diagnosis algorithms.

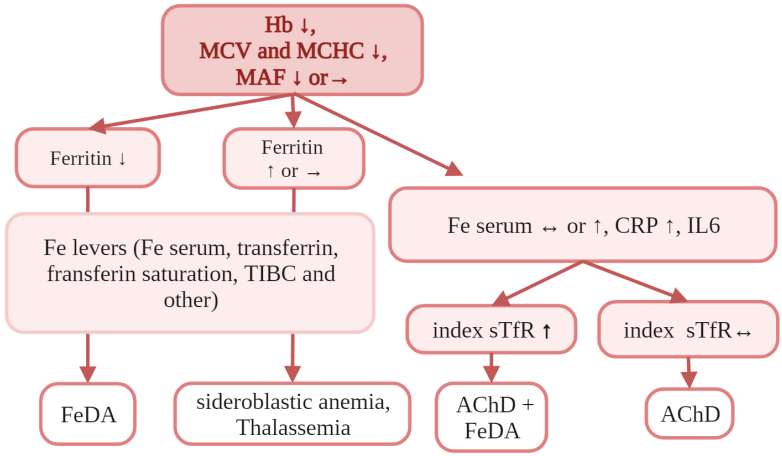

Fig. 3. Iron deficiency anemia (IDA) and Anemia of Chronic diseases (ACD)

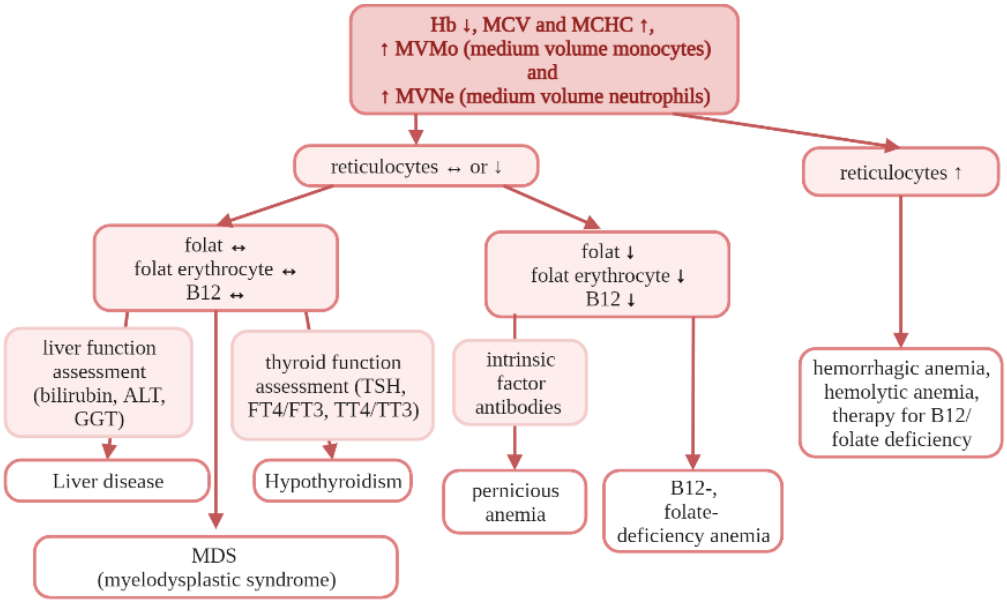

Fig. 4. Vitamin deficiency anemia (macrocytic, hyperchrome)

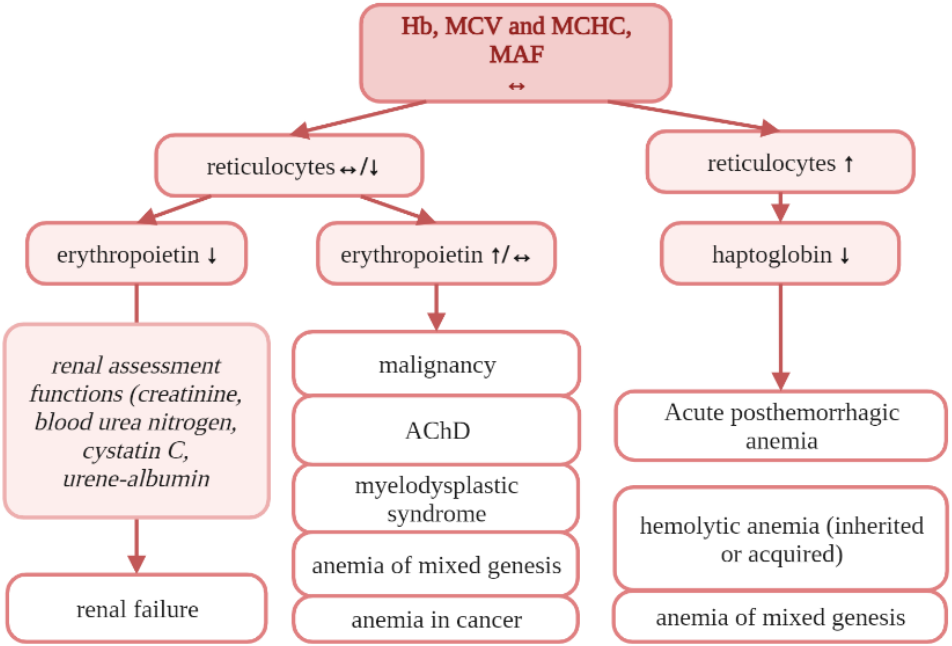

Fig. 5. Anemia for kidney failure and anemia for cancer

Hemolytic anemia assessment

The determination of the haptoglobin level is used for the diagnosis of intra- and extravascular hemolysis, acute inflammatory diseases and for differential diagnosis.

The quantification of haptoglobin in the blood is used to identify and assess the degree of hemolytic anemia and differential diagnosis of it from anemia caused by other causes. Normal haptoglobin levels are 450—1650 mg/l. A level below 450 mg/L can mean that red blood cells die faster than usual (normally 1 % of circulating red blood cells die daily, the period of life of red blood cells is 120 days).

Haptoglobin binds hemoglobin, which enters the blood when red blood cells are damaged — free hemoglobin. When hemoglobin is bound, haptoglobin forms the hemoglobin-haptoglobin complex, which is absorbed by and disposed of by liver cells. This is a physiological process in which the liver returns the amino acids globin and heme iron to the body.

Megaloblastic anemias are a group of anemias for which macrocytosis and megablastic erythrocytosis are specific.

Pathogenesis of megaloblastic anemias: Vitamin B12 and folic acid are donors and acceptors of methyl groups and atoms for DNA and RNA. As a result, these types of anemia disrupt DNA replication and RNA synthesis on it; the mitotic cycle is suppressed in cells, including blood cells. Neurological syndrome is caused by the formation of functional myelosis, because in case of a lack of B12, the conversion of methylmalonic acid to succinic acid is impaired and the myelination of nerve fibers is reduced (Fig. 6).

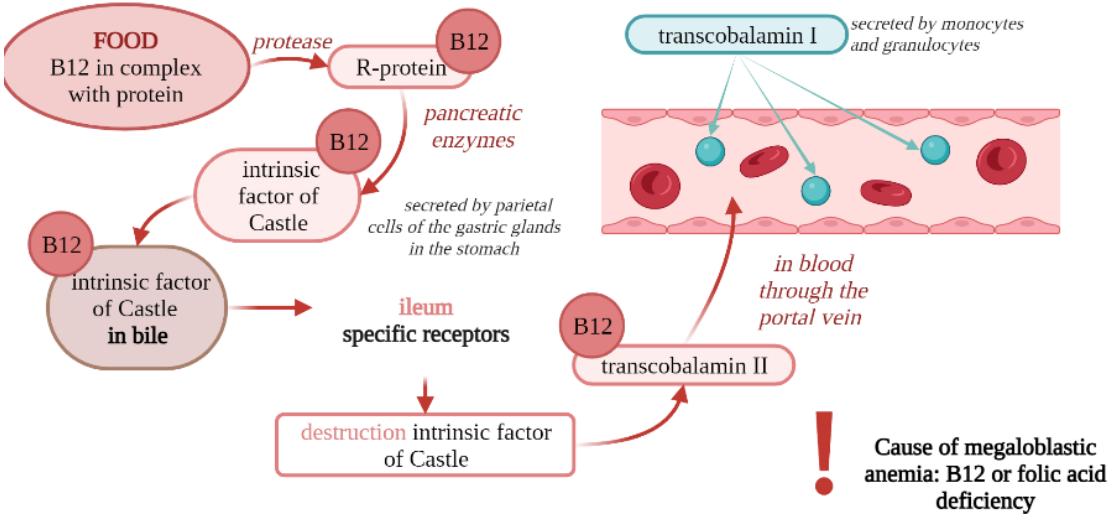

Fig. 6. Vitamin B12 metabolism

Situational tasks

Clinical example №1.

Patient, 40 years old, examined during medical examination. He made no complaints. Organ pathology, with the exception of a small increase in the liver, was not detected.

Table 3

Results of a comprehensive blood test

Test resultants		
RBC 4.48×10^6/μL	RET 0.0274×10^6/μL	HLR 0.14%
HGB 13.0 g/dl	MRV 102.5 fl	HLR 0.0062×10^6/μL
HCT 39.1%	MSCV 89.3 fl L	% MAF 11.4
MCV 87.2 fL	PLT 338×10^3/μL	WBC 6.9 10×3/μL
MCH 29.0 pg	MPV 7.7 fl	NE 50.5%
MCHC 33.2 g/dl	PCT 0.259%	LY 36.4%
RDW 13.1%	PDW 16.0	MO 8.5%
RET 0.61	IRF 0.23	EO 4.2%

Additional studies:

Mean neutrophil volume (MNV) 160 units. H — ↑ (Norm 132—144 units); mean monocyte volume (MMV) 169 units H — ↑ (Norm 157—167 units). Early manifestations of vitamin B12 or folate deficiency.

Clinical Example №2.

Female, 64, retired. 3 months ago, rheumatoid arthritis was diagnosed and anti-inflammatory treatment was prescribed. Complains of weakness and a little rapid breathing. **Examination data:** signs of RA in the joints. Pale conjunctivas. Otherwise, without features.

Table 4

Results of a comprehensive blood test

Indicators	Result	Norm
Leukocytes	5×10^9 /L	$4—10 \times 10^9$ /L
Hemoglobin	105 g/L	120—160 g/L

The end of Table 4

Indicators	Result	Norm
Hemoglobin	105 g/L	120—160 g/L
Hematocrit	32.1%	37—47%
Reticulocytes	63×10^9/L	20—80 $\times 10^9$/L
MCV (Mean Erythrocyte Volume)	**75 fL**	80—100 fL
MCH (Average Hb Content)	**23 pg**	27—32 pg
MCHC (erythrocyte saturation degree Hb)	**30 g/dL**	32—36 g/dL
RDW (degree of anisocytosis)	**15**	11—14
Platelets	230×10^9/L	150—500 $\times 10^9$/L

Stage I diagnosis — microcytic hypochromi c anemia

What additional tests will you recommend?
— ESR and C-Reactive protein.
— Iron, ferritin, transferrin, saturation transferrin.
— B12, folate.
— Oxygen pressure and oxygen saturation.
— Na, K, Ca, creatinine, uric acid.
— Check liver function (bilirubin, transaminases, albumin).
— Test serum rheumatoid factor and antinuclear antibodies.

Table 5

Results of a comprehensive blood test

Indicators	Result	Norm
Iron	**4.3 µmol/L**	5.0—30.4 µmol/L
Transferrin	1.93 g/L	1.9—2.8 g/L
Transferrin saturation	**13%**	15—50%
Ferritin	90 µg/L	11—307 µg/L
ESR	**50 mm/h**	<20 mm/h
C-Reactive Protein	**115 mg/L**	<10 mg/L

Stage II diagnosis — what is the cause of anemia?

— Anemia of chronic diseases (ACD)
— Iron deficiency anaemia (JDA)
— ACD+JDA

Additional tests are needed for confirmation

Soluble transferrin receptor reflects cell iron demand: 5.6 mg/L (0.85—3.05).

What is the interpretation of this result?

— Confirmation of chronic disease anemia.
— Iron deficiency anemia associated with chronic disease anemia.

Final diagnosis: ACD+JDA

There are four main mechanisms in the pathogenesis of ACH:

1) impaired iron metabolism;
2) erythropoiesis suppression;
3) inadequate erythropoietin production;
4) hemolytic process.

Answer: as a result of impaired iron reutilization from macrophages, the turnover and use of iron fail, so iron therapy is often ineffective. It is justified only with a concomitant iron deficiency state. The parenteral route of administration of drugs is also excluded, since iron accumulation in macrophages occurs and its supply to the bone marrow is difficult. Erythropoiesis depression is associated with exposure to inhibitors and the presence of cytokines that suppress erythropoietin (EPO).

Of great importance today is hepcidin, a protein, a regulator of iron metabolism synthesized in the liver. Hepcidin was named for its bactericidal properties. Hepcidin production in the liver depends on the body's iron stores, endogenous EPO activity, and inflammation activity. In the case of iron deficiency, the hepcidin level should be reduced to release the iron from the depot into circulation through ferroportin, which is the molecular target of hepcidin. The release of iron from the cell is blocked by the binding of hepcidin to ferroportin, which is immersed in the cytoplasm and destroyed

by the cell's lysosomes. Iron-overloaded macrophages, which are common in chronic inflammation, also interact with the hepcidin-ferroportin complex. As a result of the blockade of ferroportin, the exit of iron from macrophages is difficult. With anemia and hypoxia, there is a decrease in the expression of the hepcidin gene, which leads to an increase in iron uptake from both macrophages and the intestines. However, limiting the functional availability of iron and reducing the biological activity of erythropoietin lead to a decrease in erythropoiesis and the development of anemia. Anemia in ACH worsens as the disease increases. Iron metabolism dysregulation is a pathophysiological sign of ACH with increased iron intake and retention by RES cells. This leads to the shutdown of iron from the exchange in the RES depot, followed by the restriction of the suitability of iron for erythrocyte progenitor cells and iron-restrictive erythropoiesis.

Clinical Example №3.

Woman 72 years old, 62 kg, weight loss: 4 kg in 8 months. Complaints of weakness, loss of appetite, not previously observed neurological symptoms (paresthesia of the limbs). On examination: pallor, tachycardia. Otherwise, without features.

Table 6

Results of a comprehensive blood test

Indicators	Result	Norm
WBC	**3.5×10^9/L**	4—10×10^9/L
RBC	**2.9×10^{12} /L**	3.8—5.8×10^{12}/L
Hb	**98 g/L**	12—16 g/L
Ht	**31.3%**	37—47%
MCV	**112 fL**	80—100 fL
MCH	29 pg	27—32 pg
MCHC	33 g/dL	32—36 g/dL
RDW	**16**	11—14
Reticulocytes	**15×10^9**	20—80×10^9/L
Platelets	**132×10^9/L**	150—350×10^9/L

Stage I diagnostics

macrocytic anemia + moderate pancytopenia

Stage II diagnostics

What additional tests will you recommend?
— ESR and C-Reactive protein
— Iron, ferritin, transferrin, transferrin saturation
— B12, folate
— Oxygen pressure and oxygen saturation
— Na, K, Ca, creatinine, uric acid
— Check liver function (bilirubin, transaminases, albumin)

Table 7

Results of a blood test

Index	Result	Norm
Folate	7.5 nmol/L	>6.8 nmol/L
B12	**75 pmol/L**	75 pmol/L

Treatment for B12 deficiency was prescribed.
Tablets B12 (1,000 mcg/d).
A month later — anemia and B12 deficiency are not eliminated
What do you suggest?
• Gastroscopy
• Internal factor antibodies (IFAb)

Table 8

Result of an additional test

Setting	Result	Norm
IFAb	**1.80** AU/mL	<1.52 AU/mL

Final diagnosis: pernicious anemia

Clinical Example №4.
Patient B., 81 years old, was admitted to the hematology department with complaints of general weakness, dizziness, shortness of breath at rest, jaundice of the skin, darkening of urine. Skin, mu-

cous membranes pale, icteric, clean, normal humidity. The sclera is icteric. Musculoskeletal system with no apparent pathology. Lymph nodes are not enlarged.

Table 9

Clinical laboratory results

Index	Result	Norm
WBC	**13.5×10^9/L**	$4—10\times10^9$/L
RBC	**1.38×10^{12}/L**	$3.8—5.8\times10^{12}$/L
Hb	**6.3 g/L**	12—16 g/L
Ht	**17.7%**	37—47%
MCV (Mean Erythrocyte Volume)	**128.3 fL**	80—100 fL
MCH (Average Hb Content)	**19 pg**	27—32 pg
MCHC (Erythrocyte Hb Saturation Degree)	**23 g/dL**	32—36 g/dL
RDW	12	11—14
Reticulocytes	**255×10^9**	$20—80\times10^9$/L
Platelets	241×10^9/L	$150—350\times10^9$/L

RDW (Red cell Distribution Width) is an indicator of the distribution of red blood cells by volume, reflecting the degree of variability in the volume of red blood cells.

Red blood cell morphology. Normoblasts 3 per 100 cells.

Table 10

Clinical laboratory results

Indicatorsb	Result	Norm
Total Protein	57.5 g/l	64—84 g/l
Albumin	44.4 g/l	35—53 g/l
Globulin	13.1 g/l	48—80 g/l
Albumin-globulin coefficient	3.4 c.u.	1—2 c.u.

The end of Table 10

Indicatorsb	Result	Norm
Urea	7.0 mmol/L	In men under the age of 50 it is 3.2—7.3 mmol/l, in women — 2.6—6.7 mmol/l
Creatinine	90.0 mmol/L	53 to 97 mmol/L in women, 62 to 115 mmol/L in men
Uric acid	0.56 mmol/L	0.16—0.4 mmol/L in women and 0.24—0.60 mmol/L in men
Total bilirubin	132.2μM/L	0—21 μmol/L.
Bilirubin Direct	32.2 μM/L	2 — 5.5 μmol/L.
Iron	36.2 μM/L	9 to 30 μmol/L for women and 12 to 31 μmol/L for men.

Immunohematology: 0 (I) blood group, Rh (+) positive, Kell reaction positive.

The Kell blood group system (also known as the Kell-Cellano system) is a group of antigens on the surface of red blood cells that are important determinants of blood and serve as a target for many autoimmune or alloimmune diseases that destroy red blood cells.

Answer. Haemolytic anaemia was diagnosed. The patient underwent prednisolone therapy with a good positive effect in the form of I ncreased hemoglobin levels.

Treatment. Prednisolone 120 mg i.v. cap, 9 mg on 90-day, 13-day transfer to tablets 30 mg. Then, a dose reduction of 5 mg per day with a good positive effect in the form of an increase in hemoglobin level.

List of recommended references

1. *Butlitsky D.M*. Differential diagnosis of anemia. Scientific and practical seminar for specialists of coinical laboratory diagnostics of healthcare institutions. St. Petersburg, 2017. P. 1—54.

2. *Butler L.I*. Algorithms for the diagnosis and treatment of anemia // Russian Medical Journal. 2003. №8. P. 427.

3. *Botvinyeva V.V., Gordeeva O.B., Namazova-Baranova L.S.* Prospects for the diagnosis and treatment of various types of anemia in children // Pediatric pharmacology. 2012. №9. P. 35—40.

4. *Andreichev N.A.* Differential diagnosis of anemias not related to iron exchange // Russian Medical Journal. 2016. №22. P. 259—266.

5. *Novitsky V.V., Urazova O.I.* Pathophysiology. M. : GEOTAR-Media, 2018. Vol. 2. 592 p.

6. *Novitsky V.V., Goldberg E.D., Urazova O.I.* Pathophysiology. M. : GEOTAR-Media, 2015. 4th ed. Vol. 1. 848 s.

7. *Grossman S., Porth C.* Porth's pathophysiology concepts of altered health states // Lippincott Williams & Wilkins. 2013. 9th ed. P. 1660.

8. *Copstead L.E., Banasik J.L.* Pathophysiology // Saunders. 2012. 5th ed. P. 1216.

TOPIC 2

PATHOPHYSIOLOGY OF WHITE BLOOD

Purpose:

Study modern ideas about the causes and mechanisms of the occurrence and development of white blood pathology, as well as about the mechanisms of development of the main clinical manifestations of white blood pathology.

It is necessary to:

— have an idea of the main forms of white blood pathology;

— know the etiology, pathogenesis and manifestations of typical white blood disorders;

— be able to solve typical situational tasks and test tasks on the topic of the lesson;

— have the skill to use data from peripheral blood tests, in some cases bone marrow as diagnostic and prognostic criteria in patients with white blood pathology.

Cytopenic syndrome (cytopenia) — a decrease in the content of certain form elements in peripheral blood due to the suppression of cell development in the bone marrow (erythrocytes, megakaryocytes or leukopoieesis hematopoietic lineage in isolation and in different combinations) or increased breakdown of blood cells with sufficient production. It is characterized by the development of anemia and thrombocytopenic syndromes. It can manifest itself as leukopenia, agranulocytosis. The total decrease in the content of all shaped elements in peripheral blood is considered as pancytopenia.

Leukopenia should be considered the level of white blood cells, which with repeated blood tests (at least three) is on average less than 4.0×109/l. At the beginning of the diagnostic search for unclear leukopenia, you must have:

• Repeated blood tests with mandatory reticulocyte and platelet counts;

• Information on liver sizes — determine its percussion dimensions according to Kurlov;
• Spleen palpability information;
• Urinalysis with hemosiderin
• Blood chemistry for bilirubin and fractions, ALT, AST, prothrombin, cholesterol (liver function).

Classification of leukopenia by pathogenesis

1. ***Leukopenia due to impaired white blood cell flow from red BM of blood.***

Mechanisms:

• Damage to hematopoietic cells. Myelotoxic leukopenia develops (Fig. 1).

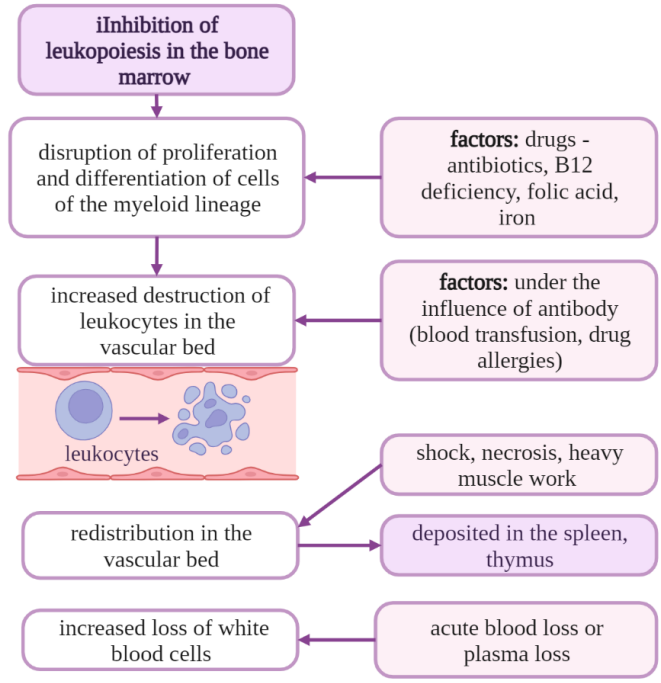

Fig. 1. Mechanism of development of leukopenia

Three main mechanisms of damage to hematopoietic cells are distinguished:

1. *Cytolytic mechanism.* It is associated with the effect on cells of ionizing radiation, cytostatics, immune factors (antibodies, T-lymphocytes, etc.). The degree of damage to BM in this case depends on the dose and duration of action of these factors.

2. *Antimetabolic mechanism.* It is based on the action of agents that interfere with the exchange of purine and pyrimidine bases, while disrupting the processes of stem cell division. According to this principle, some antitumor drugs and antibiotics (levomycetin) operate.

3. *Idiosyncratic mechanism*. It is realized with repeated administration of drugs, the sensitivity of the body to which is increased (idiosyncrasy). More often these are drugs containing benzene rings in their structure. In the case of idiosyncrasy, there is no association between the likelihood of leukopenia and dose, as well as the duration of action of drugs.

• Mitosis disorders — ineffective leukopoiesis.

Most often, its cause is:

— Deficiency of substances necessary for cell division (in particular, deficiency of vitamin B12 and folic spit),

— Dysregulation of mitosis — leukopoietin deficiency (pathology of cells forming the so-called hematopoietic growth factors and cytokines — GM-CSF, G-CSF, IL-3, M-CSF, etc.).

• Impaired white blood cell maturation.

They can be based on genetically determined defects in both hematopoietic cells themselves (for example, Costman's neutropenia) and microenvironment cells (for example, leukopenia in «steel» mice of the SL/SLα line). In this case, the maturation of blood cells reaches a certain stage (for example, promyelocytes) and stops.

• Disorders of white blood cell release from red BM into blood. Such disorders are often associated with genetic defects that disrupt the basic properties and functions of white blood cells (for example, mobility). Examples are «lazy» white blood cell syndrome, neutropenia of «Yemeni Jews».

• Reduction of leukopoiesis bridgehead. It takes place during the replacement of hematopoietic tissue with leukemia cells, metastases of tumors, etc.

2. ***Leukopenia due to reduced white blood cell residence time in peripheral blood.***

Mechanisms:

• Leukocyte destruction may be due to:

— Autoimmune mechanism (Systemic Lupus Erythematosus (SLE), rheumatoid arthritis);

— Under the influence of antibodies such as leukoagglutinins formed during blood transfusion (especially leukocyte mass);

— Hapten mechanism (drug neutropenia) — sulfanilamides,

— Action of toxic factors of infectious origin (severe infectious diseases, extensive inflammatory processes);

— Hypersplenism (increased phagocytes activity of spleen macrophages in collagenosis, cirrhosis, hemolytic anemia).

• Increased use of white blood cells. This is preceded by an accelerated release of white blood cells from the blood into the tissue under conditions of chronic recurrent inflammation.

• Enhanced elimination of white blood cells from the body. A pronounced chronic loss of neutrophils is observed in smokers: during the morning cough with sputum, 0.5 to 2×10^8 granulocytes and 0.8 to 1.6×10^8 macrophages are lost. Loss of white blood cells is characteristic of purulent endometritis, cholecystocholangitis, etc.

3. ***Redistributive leukopenia.***

The ratio between circulating and wall (marginal) pools of white blood cells change as a result of their accumulation in expanded capillaries of depot organs (lungs, liver, intestines). Observed in shock, neuroses, inflammatory diseases, malaria. This leukopenia is temporary and is usually replaced by leukocytosis.

If the liver is reduced, then the detected leukopenia (in the early stages) should be associated with cirrhosis of the liver. A detailed study of biochemical parameters (prothrombin and cholesterol are often reduced), esophageal veins, liver biopsy allows you to confirm or refute **cirrhosis**. If leukopenia is detected against the back-

ground of urinary syndrome inherent in **chronic nephritis or pyelonephritis**, then the corresponding pathology must be accurately verified.

Situational tasks

Clinical Example №1.

Patient, 30 years old, with complaints such as sore throat, cough, chills, weakness and difficulty swallowing (catarrh of the upper respiratory tract). No organ pathology was detected — the liver was not reduced in size; the spleen was not enlarged. Repeated blood tests show normal indicators of platelets and red blood cells. Results of a comprehensive blood test:

Table 1

Clinical and laboratory findings

Indicators	Result	Norm
Leukocytes	**3×10^9/L**	$4—10\times10^9$/L
Erythrocytes	3.83×10^{12}/L	$3.8—5.8\times10^{12}$/L
Hemoglobin	**105 g/L**	12—16 g/dL
Hematocrit	**32.1%**	37—47%
Reticulocytes	63×10^9/L	$20—80\times10^9$/L
MCV	85 fL	80—100 fL
MCH	28 pg	27—32 pg
MCHC	34 g/dL	32—36 g/dL
RDW	12	11—14
Platelets	230×10^9/L	$150—350\times10^9$/L

If the liver is not reduced, the spleen is not enlarged; repeated blood tests detect normal values of platelets, red blood cells, the number of white blood cells is higher than $2—3\times10^9$/L — this is a redistributive leukopenia, often found in viral infection. An additional feature may be the detection of a small number of lymphocytes with a wide basophilic cytoplasm having perinuclear enlightenment. After 1—2 months, leukopenia disappears.

Clinical Example №2.

Leukopenia in a person who feels sick (Fig. 2).

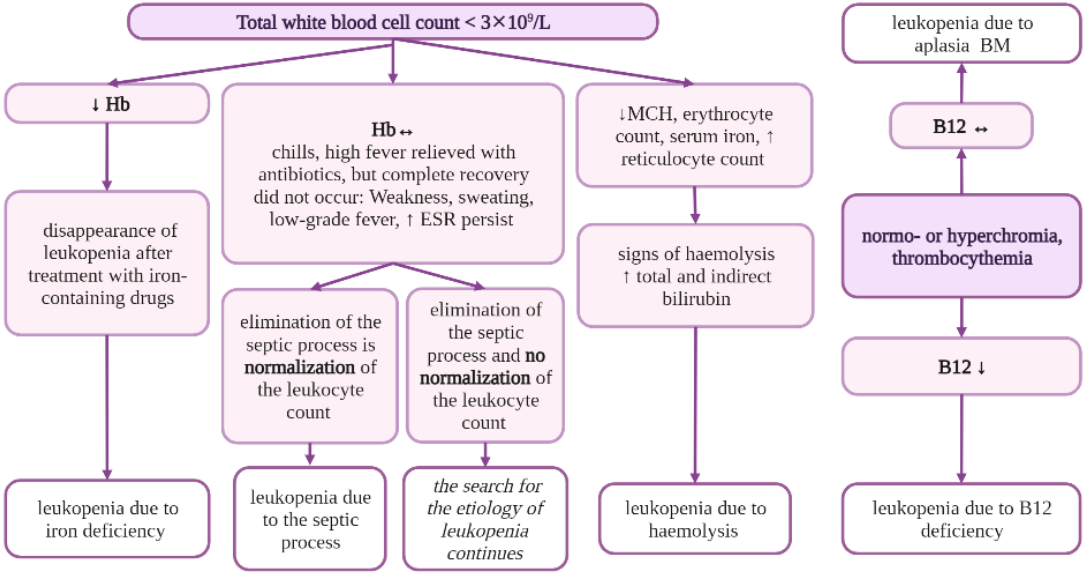

Fig. 2. Leukopenia

Clinical Example №3.

The patient presents complaints of persistent or periodic malaise. White blood cell level is $2—4 \times 10^9$/L (in most tests, the blood formula is unchanged or relative lymphocytosis due to neutropenia). Red blood and platelet count were normal and there were no biochemical abnormalities (Fig. 3).

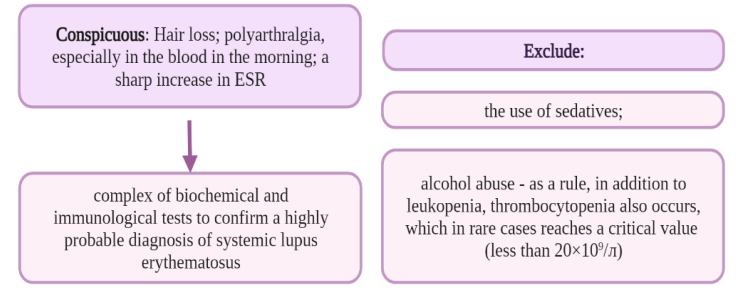

Fig. 3. Diagnostic algorithm

Agranulocytosis

A clinical and immunological syndrome characterized by a sharp decrease (less than 0.75×10^9/L) or complete disappearance of granulocytes in peripheral blood and a total number of white blood cells less than 1.0×10^9/L.

Causes of agranulocytosis:

• Ionizing radiation and radiation therapy, chemicals (benzene), insecticides.

• Drugs can cause agranulocytosis as a result of direct suppression of hematopoiesis (cytostatics, valproic acid, carbamazepine, beta-lactam antibiotics), or acting as haptens (gold preparations, antithyroid drugs, antituberculosis, etc.).

• Autoimmune diseases (e. g., Lupus erythematosus, autoimmune thyroiditis).

• Viral infections (caused by Epstein-Barr virus, cytomegalovirus, viral hepatitis) are usually accompanied by moderate neutropenia, however agranulocytosis may develop in some cases.

• Severe generalized infections (both bacterial and viral).

Manifestations of agranulocytosis:
• Necrotic sore throat.
• Ulcerative necrotizing stomatitis.
• Pneumonia (often abscessing).
• Necrotic enteropathy.
• Sepsis.

Differential diagnosis should be done with aplastic anemia and acute leukemia.

Leukocytosis

Table 2

Changes in the leukocyte count depending on age and pregnancy

Patient Profile	Normal total white blood cell count
Newborn baby	13.0 to 38.0×10^9/l
Two-week-old infant	5.0 to 20.0×10^9/l
Adult	4.5 to 11.0×10^9/l
Pregnant woman (third trimester)	5.8 to 13.2×10^9/l

Table 3

Adult leukocyte formula

Adult leukocyte formula						
Total white blood cell count	Types of leukocytes and their number, %					
	Neutrophils		Eosino-phils	Baso-phils	Mono-cytes	Lympho-cytes
	band	segmented				
4—9×10^9/L	2—5	43—65	2—5	1	6—9	27—45

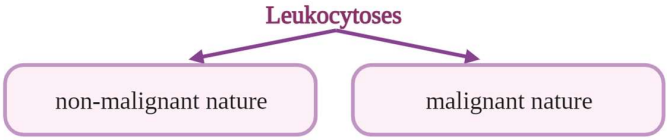

Fig. 4. Types of leukemia of various natures

Table 4

Leukocytosis of a non-malignant nature

Morphological type of leukocytosis	Etiology of leukocytosis
Basophilia (absolute number of basophils more 0.1×10^9/l	Allergic conditions, leukemias
Eosinophilia (absolute number of eosinophils greater than 0.5×10^9/l	Allergic conditions, dermatological conditions, eosinophilic esophagitis, idiopathic hyper eosinophilic syndrome, malignancies, drug reactions, parasitic infections
Lymphocytosis (absolute lymphocyte count greater than 4.5×10^9/l	Acute or chronic leukemia, hypersensitivity reaction, infections (viral, pertussis)
Monocytosis (absolute monocyte count greater than 0.88×10^9/l	Autoimmune diseases, infections (Epstein-Barr virus, fungal, protozoal, rickettsioses, tuberculosis), splenectomy, chronic infections, endocarditis, inflammatory conditions, autoimmune diseases, granulomatous diseases, malignancies, drug side effects and myeloproliferative diseases.
Neutrophil leukocytosis (absolute number of neutrophil's greater than 7.0×10^9/l	Bone marrow stimulation, chronic inflammation, congenital, infectious, drug-induced, reactive, splenectomy

Table 5

Non-malignant neutrophilia

Reason	Distinctive features	Evaluation
Infectious disease	Fever, systemic symptoms Physical Exam Results	Obtain system-specific culture and imaging data (e.g., sputum culture,Chest X-ray). Consider empirical antibiotics. Consider using other biomarkers, such as CRP and procalcitonin

The end of Table 5

Reason	Distinctive features	Evaluation
Reactive neutrophilia	Exercise, physical stress (e.g., postoperative, febrile seizures), emotional stress (e.g., panic attacks), smoking	Confirm with medical history
Chronic inflammation	Rheumatic diseases, inflammatory bowel diseases, granulomatous disease, vasculitis, chronic hepatitis	Analysis of personal and family medical history Take into account the erythrocyte sedimentation rate and CRP levels, special rheumatologic tests. Consultation with a specialist (e.g. rheumatologist, gastroenterologist)
Medication	Corticosteroids, beta-agonists, lithium, epinephrine, colony-stimulating factors, NSAIDs, common antibiotics (nitrofurantoin, quinolones, cephalosporins, penicillin's, sulfonamides)	Analysis of medical history; Consider discontinuing or substituting medication if warranted
Bone marrow stimulation	Hemolytic anemia, immune thrombocytopenia, restoration of bone marrow suppression, colony-stimulating factors	Complete differential blood count; Compare with original values (if any). Peripheral smear examination. Reticulocyte and lactate dehydrogenase analysis. Flow cytometry analysis, bone marrow examination, consultation with a hematologist / oncologist
Splenectomy	History of trauma or sickle cell anemia	Analysis of medical history
Connatural	Hereditary/chronic idiopathic neutrophilia, Down syndrome, leukocyte adhesion failure	Get information about family history, medical history. Consultations in Hematology / Oncology, Genetics and Immunology

Absolute lymphocytosis: acute infections — cytomegalovirus, Bordetella pertussis, hepatitis, toxoplasmosis and Epstein-Barr virus; chronic infections — brucellosis and tuberculosis.

Basophilia can occur in inflammatory conditions, viral infections, endocrinopathies, myeloproliferative diseases, and malignancies. Like eosinophils, it is also present in allergic or anaphylactic conditions, especially in reactions to drugs and food. This is a relatively rare cause of leukocytosis. Transient hyperbasophilia can occur as a reactive response, especially to acute viral disease. Persistent basophilia present in multiple complete blood counts for more than eight weeks is indicative of malignancy or myeloproliferative disease, and leukemias associated with basophilia are extremely rare.

Situational tasks

Clinical case of non-malignant neutrophilia №4.

Neutrophilic postsplenectomy with sepsis. The 18-year-old young woman, who had undergone distal pancreatic resection and splenectomy for a benign pseudopapillary pancreatic tumor two weeks earlier, was admitted to the emergency department with fever (39.4 °C), leukocytosis, tachycardia, and urine test results suggestive of infection. Leukocyte count 57.1×10^9 per l: myelocytes — 5, myelocytes — 8, band neutrophils — 12, segment eutrophils — 49, lymphocytes — 25; Hb 126 g per l, platelet count 832×10^9/l.

Leukocytoses of malignant nature:

Neutrophilia — chronic myeloid leukemia, myeloproliferative neoplasms, myelodysplastic syndromes, acute myeloid leukemia, malignant neoplasms of the gastrointestinal tract or kidneys, melanoma, Hodgkin's disease.

Lymphocytosis — lymphoma, leukemia.

Monocytosis — Hodgkin's disease, multiple myeloma, acute myeloid leukemia, myeloproliferative neoplasms, myelodysplastic syndromes, including chronic myelomonocytic leukemia.

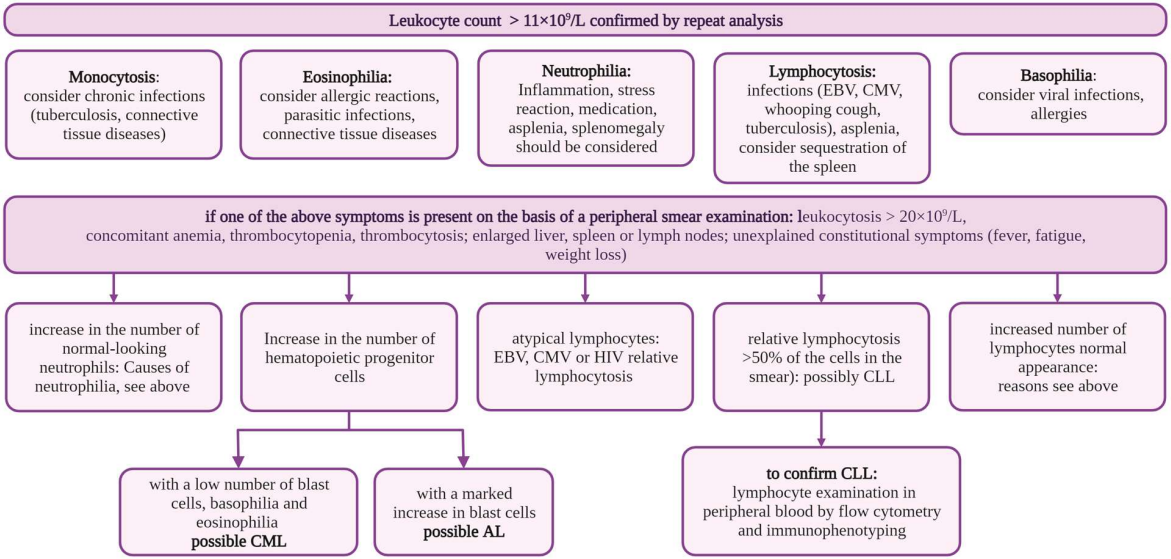

Fig. 5. Risk assessment in Leukemia

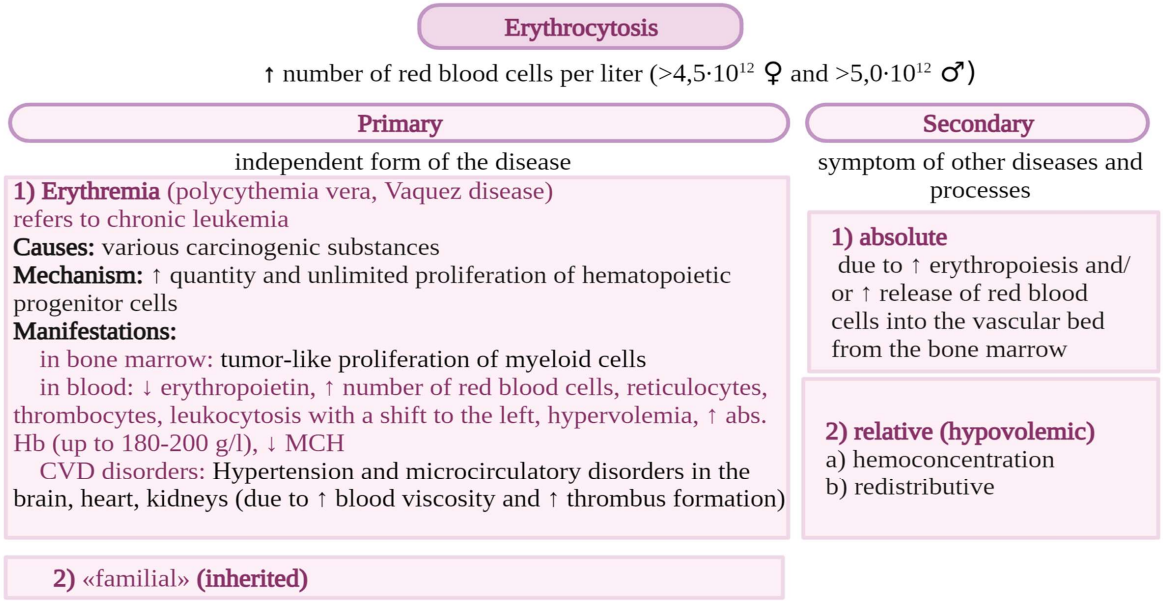

Fig. 6. Classification of erythrocytosis

Traditionally, all leukemias are divided into acute (AL) and chronic (ChL). The distinction between AL and ChL is based on the ability of the tumor cells to differentiate (mature). A chronic variant of leukemia is diagnosed when the tumor cell is capable of differentiation at a certain stage of the disease. Acute leukemia is diagnosed when a primary mutated hematopoietic cell is not capable of differentiation but can only proliferate itself, leading to the accumulation of a large number of immature blast cells and suppression of normal hematopoiesis. «The tumor growth of all hemoblastoses is based on ***clonality*** — every leukemia or hematosarcoma owes mutations in its mother cell to the entire mass of its cells» — A.I. Vorobyov.

Etiology of hemablastoses:

• Ionizing radiation, radiation therapy (20—40 rad);

• Chemical mutagens (cytostatic preparations mustargen, cyclophosphane, etc.); for example, secondary leukemias after treatment of lymphogranulomatosis with a combination; radiotherapy and mustargen;

• Viruses (Epstein-Barr — Burkitt lymphoma). The virus is a stimulant;

• Heredity.

Pathogenesis of hemoblastoses:

• Clonality: monoclonal and polyclonic stages;

• Tumor progression;

• Inhibition of hematopoietic sprouts;

• Metastasis;

• Resistance to therapy.

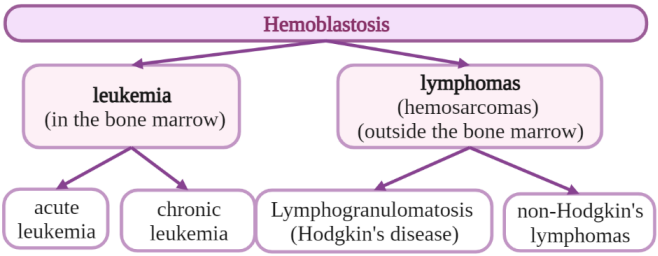

Fig. 7. Classification of hemoblastosis

Principles of Clinical and Laboratory Diagnostics of Leukemia

1. Features of the clinical picture:

• Intoxication syndrome (more pronounced in acute leukemia);

• Anemic (circulatory-hypoxic syndrome);

• Lymphoproliferative syndrome (lymphadenopathy and splenomegaly — more common in lymphoid leukemia);

• Hemorrhagic syndrome;

• Infectious syndrome (fever, necrotic sore throats, septic condition);

• Skin lesions (leukemids);

• Neuroleukemia (paralysis, paresis, coma of unclear origin) —more common in lymphoblastic leukemia;

• Defeat of the Gonads.

2. Changes in peripheral blood suspected of leukemia:

• Hypoplasia of 2 hematopoietic lineages (anemia and thrombocytopenia, leukopenia and thrombocytopenia, leukopenia and anemia);

• Leukocytosis greater than 20×10^9/l in the absence of signs of an obvious infectious process;

• Lymphocytosis >48%;

• Concomitant erythrocytosis, hemoglobinemia, leukocytosis, thrombocytosis;

3 Clinical blood count in AL:

• *Anemia* detects usually normochromic (a decrease in hemoglobin below 100 g/l) in 85% of patients.

• *Leukopenia* is less than 4×10^9/l in 20%, and leukocytosis is more than 10×109/L in 50% of patients. Blast cells in the hemogram may be absent (aleukemic stage), can be found in small numbers (3—5% subleukemic stage), or make up the bulk of the cell population (leukemic form).

• *Thrombocytopenia* less than 100×10^9/l in 80% of patients.

• In almost all patients (98%), *blast cells* are detected in the peripheral blood.

• Characteristic is the so-called «leukemic gaping»: In the leukocyte formula of an AL patient, blast forms are present and then, without the presence of transitional forms (promyelocytes and myelocytes), mature granulocytes, the number of which may be significantly reduced due to an increased proportion of lymphocytes or blast cells.

4. Myelogram analysis.

In bone marrow more than 30% of blast cells (with the exception of acute low-interest leukemia, in which for many months in the blood and bone marrow of blast cells can be less than 15—20%, and in the bone marrow in this form, as a rule, the percentage of blasts is less than in the blood), in peripheral blood — cytopenia up to pancytopenia. Therefore, in cytopenia, even concerning one sprout, a bone marrow puncture is necessary, which can be done on an outpatient basis.

Table 6

Classification of leukemia
(FAB, 1976 — French—American—British classification)

Non-lymphoblastic (Myeloid)	Lymphoblastic
M0 — acute myeloid leukemia without signs of maturation	**L1.** *Lymphoblasts* are typical microlymphoblasts with sparse cytoplasm
M1 — acute myeloid leukemia with minimal signs of maturation	
M2 — acute myeloid leukemia with maturation	**L2.** *Lymphoblasts* are large with an abundance of moderately basophilic cytoplasm, distinct 1 to 3 nucleoli, most of which are irregularly shaped
M3 — acute promyelocytic leukemia	
M4 — acute myelomonocytic leukemia	
M5a — acute monoblastic leukemia without differentiation	**L3.** *Lymphoblasts* are cells with pronounced cytoplasmic basophilia and often with its vacuolization
M5b — acute monoblastic leukemia with differentiation	
M6 — acute erythroid leukemia	
M7 — megakaryocytic leukemia	

Diagnosis of acute leukemia

- Clinical blood test + morphology
- Bone marrow cell morphology (bone marrow punctate)
- Bone marrow histology (trepanobiopsy)
- Immunohistochemical studies

Table 7

Chronic leukemia

Myeloproliferative leukemias	Lymphoproliferative leukemia (T and B)
Chronic myelogenous leukemia	B-chronic lymphocytic leukemia
Polycythemia vera	Non-Hodgkin lymphomas
Idiopathic thrombocythemia	Paraproteinemic hematological malignancies • Myeloma • Waldenstrom macroglobulinemia • Heavy Chain Diseases
Subleukemic myelosis	

Features of chronic leukemia.

• Unlike acute, in chronic leukemia, blood cells manage to «mature», but the vast majority of the resulting blood cells are unable to fulfill their direct function.

• In 50% of patients with chronic leukemia, there are no complaints about their own health, they are diagnosed with leukemia according to a blood test performed for another reason.

• Even after diagnosis, chronic leukemia can flow for years, relatively benign.

• *Monoclonal* phases are characterized by the presence of a single clone of tumor cells.

Polyclone stage (blast crisis) — due to the appearance of secondary tumor clones, characterized by rapid flow with the appearance of many blasts.

• 80% of chronic leukemia patients die during blast crisis.

List of recommended references

1. *Differential* diagnosis in cytopenic syndrome / ed. L.A. Panchenkova. Educational and methodological manual. M., 2018.

2. *Riley L.K., Rupert J.* Evaluation of Patients with Leukocytosis // Am Fam Physician. 2015. Vol. 92, №11. R. 1004—1011.

3. *Mank V., Azhar W., Brown K.* Leukocytosis // StatPearls, National Library of Medicine. 2023. URL: https://www.ncbi.nlm.nih.gov/books/NBK560882/ (accessed 04.12.2023).

4. *George T.I.* Malignant or benign leukocytosis // Hematology Am Soc Hematol Educ Program. 2012. Vol. 2012. P. 475—484.

5. *Mezei G., Chang E.T., Mowat F.S. et al.* Comments on a recent case-control study of malignant mesothelioma of the pericardium and the tunica vaginalis testis // Scand J Work Environ Health. 2021. Vol. 47, №1. R. 85—86.

6. *Novitsky V.V., Urazova O.I.* Pathophysiology. M., 2018. Vol. 2.

7. *Vorobyov S.A*. Hemoblastosis. Acute and chronic leukemia. Modern principles of diagnosis and therapy // Department of Faculty Therapy. 2018. URL: https://documen.site/download/11669_pdf# (accessed 04.12.2023).

8. *Porth C., Grossman S.* Porth's pathophysiology concepts of altered health states // Lippincott. 2013.

9. *Copstead L.E., Banasik J.* Pathophysiology // Saunders. 2012.

TOPIC 3

PATHOLOGY OF THE HEMOSTATIC SYSTEM

The purpose of the session: To form modern ideas about the types and mechanisms of disorders of vascular-platelet and coagulation hemostasis. Determine the main changes in the physicochemical properties of blood. Introduce students to modern methods of detecting hemostasis pathology.

It is necessary to:

— Have an idea of the main types of hemostasiological disorders.

— Know the mechanisms of violations of the coiling and anti-coiling systems.

— Be able to solve typical situational tasks and test tasks on the topic of the lesson.

Thrombophilia

It is important to understand the ambiguity of the interpretation of the terms «thrombophilia», «thrombogenic risk factors» and «hypercoagulation syndrome or condition». The carriage of a known prothrombogenic mutation or polymorphism of genes (participants in hemostatic reactions and methionine exchange) is often considered and diagnosed as thrombophilia, leading to overdiagnosis (!), causing moral harm to patients and their relatives (Fig. 1).

According to the ISTH recommendations adopted in Sapporo (1998) and Sydney (2006), the diagnosis of antiphospholipid syndrome is considered reliable when at least one or more clinical manifestations of this pathology (vascular thrombosis, pregnancy pathology) are combined with the results of special laboratory tests (effects of lupus anticoagulant, AFA in the diagnostic titer) (Table 1).

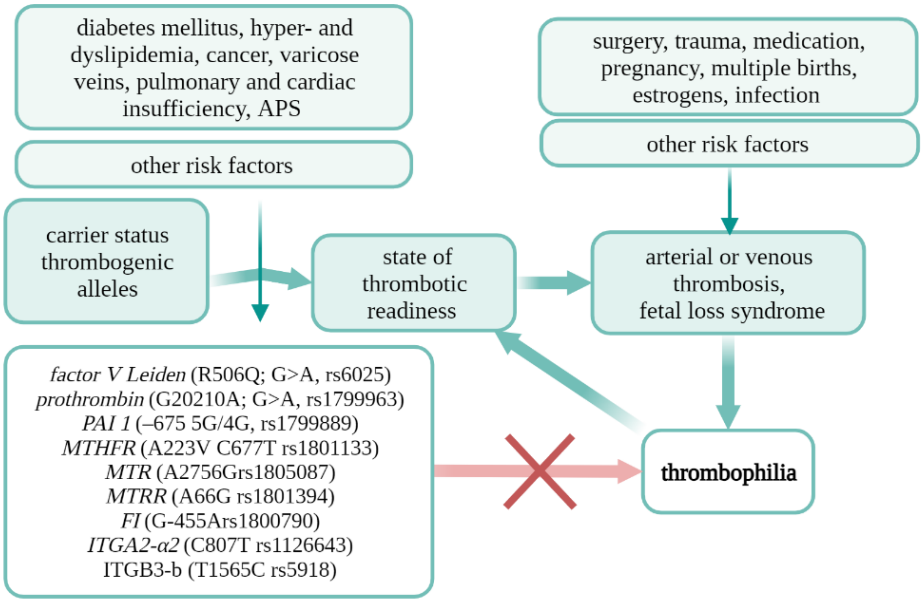

Fig. 1. Thrombogenic risks

Table 1

Antiphospholipid syndrome (APS) — Diagnosis

Clinical Criteria	Laboratory Criteria
1. Vascular thrombosis: — One or more cases of arterial and/or venous thrombosis or thrombosis of small vessels in any organ or tissue; — Thrombosis should be confirmed by Doppler examination or histology; — Morphologically, there should be signs of thrombosis without significant inflammation vascular wall	1. Anticardiolipin antibodies: — Presence of IgG and IgM isotypes in high credits of 2 or more research at intervals at least 12 weeks; — Identification of standardized ELISA IgG antibodies, IgM to β2-GPI. 2. Lupus anticoagulant. Found in 2 or more sequential studies at least 12 weeks apart
2. Pathology of pregnancy: — 3 or more unexplained cases of termination of pregnancy up to 10 weeks of gestation, with the exclusion of anatomical, genetic, hormonal causes and chromosomal abnormalities; — 1 or more cases of intrauterine death of a normal fetus after 10 weeks of gestation; — 1 or more cases of preterm birth of a preterm fetus before 34 weeks of gestation, proceeding with severe fetoplacental insufficiency or severe preeclampsia	

In addition to API, according to the same criteria, thrombophilia can include Trusso syndrome (migratory venous thromboses in the presence of a cancerous procoagulant), Moshkovich syndrome (arterial microthromboses against the background of circulation of large multimers of Willebrand factor in the presence of metallopro-

teinase ADAMTS-13), heparin-induced thrombocytopenia type 2 (subcutaneous and systemic venous thromboses in the presence of antiheparin antibodies) and a number of other pathological conditions.

Thus, the carriage of various thrombogenic risk factors without their implementation in the form of thrombosis and the fetal loss syndrome cannot be attributed to thrombophilia.

Thrombophilia is not a disease, but a pathological condition that is due to a combination of risk factors realized by the development of thrombosis (blood clots) and about which information can be obtained from the individual medical history. It may be inherited or related to a disease (e. g., cancer), medication (e. g., oral contraceptives, erythropoiesis stimulating agents) or a health condition (e. g., pregnancy, puerperium). It is essential to understand and adopt this provision because susceptibility to disease does not mean that there is an indication for primary or secondary prevention or treatment. Currently, more than 100 thrombogenic risk factors and conditions associated with thrombophilia have been described, which in combination can lead to vascular catastrophes. It is considered that the division of these factors into hereditary (congenital) and acquired is not justified, as the vast majority of diseases and abnormalities in humans are genetic. The classification of thrombogenic risk factors may be based on the duration of exposure to the human body and the controllability by the patient or on the use of modern medicine to reduce the likelihood of arterial or venous thrombosis.

Uncontrolled risk factors — age, family and personal thrombotic history, carrying thrombogenic mutations and polymorphisms, low mobility associated with severe injuries, blood groups «II—IV», systemic manifestations of angiodysplasia and some others — cannot be corrected and accompany a person throughout his life. Much more numerous are the transient and comparatively more controllable risk factors, which in turn can be subdivided into lifestyle-related factors. (e. g., bad habits, hypodynamia, stress due to mental and physical overload), individual characteristics. (pregnancy), disease-related (diabetes mellitus, arteriosclerosis, high blood pressure, cardiac arrhythmia) and iatrogenic — caused by medical interventions (surgery and the prescription of a range of

medications — see above). The controllability of these risk factors varies and should be assessed in each individual case with regard to both the etiology and the pathogenesis of the thrombosis. The following coagulation, amidolytic, immunology and genetic tests can be recommended for the laboratory detection of causes contributing to thrombosis (Table 2).

Table 2

Laboratory criteria for diagnosis of thrombogenic risk

Type of research	Detectable metric
Complete blood count	Erythrocyte and platelet count, hemoglobin level, erythrocyte sedimentation rate. Determination of blood type
General Coagulogram Screening	Prothrombin test, activated partial thromboplastin time, thrombin/reptiles time, fibrinogen concentration
Vascular-platelet Hemostasis link	Aggregation of platelets on an aggregometer with different agonists. Test for the presence of heparin-induced thrombocytopenia type 2: determination of antibodies against the heparin factor 4 complex platelets (antiheparin), assessment of heparin-dependent platelet aggregation. Factor antigen von Willebrand. ADAMTS-13 metalloproteinase activity
Coagulation link of hemostasis	Activity of coagulation factors II, VIII, IX, XI and XIII. Antibodies with the ability to prolong clotting time in phospholipid-sensitive coagulation assays, with confirmatory studies. Evaluation of polymerization (self-assembly). Fibrin monomer in the diagnosis of dysfibrinogenemia

The end of Table 2

Type of research	Detectable metric
Physiological anticoagulants	Activity and antigen of antithrombin III and protein C. Activity of total and free protein S. APS resistance. TFPI Level
Fibrinolytic activity of blood	Plasminogen, t-PA, PAI-1 and TAFI levels
Genetic investigations	Carrier of factor V Leiden mutation (1691 G/A), prothrombin mutation (20210 G/A) and a number of others (see Table 4). JAK2 tyrosine kinase (Janus kinase) mutation
Additional Studies	Antiphospholipid autoantibodies to cardiolipin and β2-GPI. Serum homocysteine level (basal and after methionine loading)

The terms «thrombophilia» and «increased blood clotting» are often used interchangeably, although they are different concepts. Increased blood coagulation or «hypercoagulation syndrome/condition» is a laboratory phenomenon in which the activation of platelets and the process of fibrin formation and, in some cases, the suppression of fibrinolytic reactions is detected *in vitro* using special analytical methods of the hemostasis system.

Momot A. P. proposes an alternative, clinically based concept — the «*thrombotic state of readiness*», which may combine laboratory evidence of hyper- or hypocoagulation, an increase in intravascular coagulation markers and a number of the clinical signs of pre-thrombosis mentioned above. If the risk factors persist and multiply (e. g., surgery, injury, inflammation, emergencies, immobilization, HF, dehydration, stress, estrogen use, etc.), the realization of this readiness manifests in vascular catastrophe. The state of thrombotic readiness thus results from the interaction of vari-

ous thrombogenic risk factors and immediately precedes thrombosis and accompanies it even in the absence or low efficacy of antithrombotic therapy (Fig. 1).

Laboratory markers of thrombotic readiness include platelet activation — according to the study of their functional activity on an aggregometer or to increase the expression of β-thrombomodulin as well as the 3rd and 4th platelets. An equally important testimony to this readiness is the increase in the concentration of a number of markers of blood coagulation activation and fibrinolysis — tissue factor, activated factor VII, thrombin-antithrombin complex, prothrombin 1+2 fragment, fibrinopeptide A, soluble fibrin monomer complexes and D-dimers. The latter play a special role, as they are used in clinical practice for the diagnosis of venous thromboembolism and to monitor the effectiveness of anticoagulants.

Situational tasks

Clinical Example №1.

Sick Sh. V., 43 years old, resident of the Altai Territory. He came to the clinic in 2005 because of a high hemoglobin level (over 180 g/L), increased blood pressure (up to 180/100 mm Hg. Art.), complaints of pressure pain in the heart, shortness of breath, palpitations and headaches. The father has a high hemoglobin level (170—190 g/L), arterial hypertension. The patient's grandfather died of an ischemic stroke. At the age of 51, the patient suffered a heart attackand suffers from transient ischemic attacks. The following parameters were determined during the examination of the patient's blood: Hemoglobin — 184 g/L, erythrocytes — 5.5×10^{12}/L, hematocrit — 48.1%. The lipidogram revealed: high cholesterol levels and low-density lipoproteins. The examination ruled out myeloproliferative diseases and symptomatic erythrocytosis due to lung, kidney and liver diseases. The homocysteine level in the blood was found to be elevated — 21.0 μmol/L. High thrombinemia markers (by the number of soluble fibrin-monomer complexes and D-dimers), an increase in platelet aggregation when assessing their function with adrenaline and collagen, a delay in XIIa-dependent fibrinolysis and an increase in the activity of factor VIII were detected in the coagulation diagram. Genetic test-

ing revealed a combination of the prothrombin (G20210A) G/A mutation with the polymorphism of *MTHGFR* (677) C/T, *PAI-1* 4G/5G, fibrinogen (–455) G/A and the platelet receptor fibrinogen GPIIIa T/C.

Conclusion. The presence of thrombogenic risk factors: carrier polymorphisms of the prothrombin (G20210A) G/A gene, *MTHFR* (677) C/T, a region of the DNA coding sequence of the *MTHFR* gene in which the base cytosine (C) can be replaced by thymine (T) at position 677 is called genetic marker S677T, *PAI-1* 4G/5G, fibrinogen and fibrinogen platelet receptor GPIIIa T/C; familial polyglobulia, hyperlipidemia, high blood pressure. Thrombosis susceptibility due to elevated levels of thrombinemia markers (soluble fibrin, D-dimers) and platelet hyperactivation.

Recommended. Conducting courses of primary thrombosis prophylaxis with the aim of normalizing homocysteine levels (taking folate-vitamin complexes, long-term); correction of hyperaggregation syndrome (antiplatelet agents after determining sensitivity to them, long-term), thrombinemia (low molecular weight heparins in preventive doses); Elimination of polyglobulia (cytapheresis, hirudotherapy); correction of hyperlipidemia with statins, selection of optimal drugs for antihypertensive therapy. Dynamic control of thrombosis readiness markers (twice a year) and, if necessary, repeat thrombosis prophylaxis.

Result. There was no increased risk of arterial ischemia in the past 10 years during which the patient was observed. The diagnosis of thrombophilia was ruled out as there were no documented clinically significant thromboses.

Clinical Example №2.

Patient M.T., 49 years old, resident of Altai Territory, was examined and consulted in November 2020 in the subacute phase of an acute cerebrovascular accident of ischemic type with damage to subcortical structures with dysarthria, tetraparesis, pelvic organ dysfunction to clarify the causes of cerebral vascular thrombosis. An aggravated maternal history of thrombosis was found. During the examination, the carrier of the polymorphism of the *MTHFR* gene (677) (a section of the DNA coding sequence of the *MTHFR* gene in which the base cytosine (C) can be replaced by thymine (T) at

position 677, referred to as genetic marker S677T) T/T in combination with hyperhomocysteinemia (22.0 μmol/L), hyperaggregation syndrome with the event of aspirin resistance, increased activity of factor VIII (210%), high thrombinemia markers (by the amount of soluble fibrin, D-dimers).

Conclusion. Thrombophilia due to the polymorphism of the *MTHFR* (677) T/T gene and an increase in factor VIII activity caused by ischemic acute cerebrovascular accident. Thrombotic condition (increased platelet activity, thrombinemia).

Recommended. Due to the development of aspirin resistance, aspirin preparations were replaced by clopidogrel. Treatment with «Angiovitis» and heparin prophylaxis with low-molecular-weight heparins was prescribed. Repeated examination for thrombotic activity — once every **2—3 months.**

Clinical example №3.

Patient N.O., 69 years old, resident in Barnaul. From 30.08. to 16.09.2010 she was in the orthopedic department of the Altai Regional Clinical Hospital with a diagnosis of idiopathic bilateral deforming coxarthrosis of the third degree. Concomitant diseases: Grade II hypertension, stage II, risk 2, chronic heart failure I, functional class II, varicose veins of the lower extremities, chronic venous insufficiency grade I. On 02.09.2010 the planned operation was performed — a total cement hip replacement. As this operation was associated with thrombosis prophylaxis, dabigatran etexilate was prescribed as thrombosis prophylaxis (per os 110 mg 4 hours after the operation, then 220 mg once daily). On the 5th day after the operation, the patient showed subclinical thrombosis of the right sural veins during a routine duplex examination of the veins of the lower extremities. Genetic examination data — mutation of factor V Leiden (1691) G/A, polymorphism of the MTHFR (677) T/T gene. A laboratory test for hemostasis revealed a high level of thrombin markers (soluble fibrin and D-dimers) after the onset of thrombosis. In addition, the Echitox test, which is recommended for monitoring the effect of dabigatran, did not show any prolongation of the clotting time, which is characteristic of the use of this direct oral anticoagulant. It was clarified that this was due to the low compliance of the patient who secretly refused to take dabigatran.

Conclusion. Thrombophilia due to factor V Leiden mutation (1691) G/A and polymorphism of MTHFR gene (677) T/T, varicose limb disease, hip replacement surgery. Thrombosis of the right sural veins in the early postoperative phase. Thrombosis readiness status due to elevated levels of thrombinemia markers (soluble fibrin and D-dimers).

Recommended. Use of enoxaparin sodium in a therapeutic dose (subcutaneous, 1 mg/kg body weight every 12 hours). Determination of homocysteine levels and platelet aggregation function. Monitoring of thrombinemia markers and duplex scanning of the veins of the lower extremities — 1 and 3 months after surgery. Decision on switching to indirect anticoagulants — vitamin K antagonists (after consultation with a psychologist and taking into account the data from the examination of the hemostasis system).

It is clear that in the various clinical examples mentioned above, the effects of a variety of permanent or transient thrombogenic risk factors are combined. However, the basis for the use of antithrombotic drugs is the presence or absence of thrombotic readiness in the patient, which is determined on the basis of objective laboratory data. At the same time, measures to correct modified or controlled thrombogenic risk factors are advisable (control of polyglobulia, hypertension, hypodynamia, hyperglycaemia, normalization of lipid metabolism, venous blood flow, avoidance of the use of oestrogens or chewable tablets, reduction of trauma during planned surgical interventions by choosing minimally invasive options, etc.).

Clinical Example №4.

A 21-year-old woman went to see a doctor because she felt unwell and had a slightly elevated temperature. She believes that she had an acute respiratory infection a week ago, after which she experienced shortness of breath with mild exertion, dizziness, weakness and elevated body temperature. On examination, rectal temperature is 37.9°C, other parameters are normal. White blood cells — 14. 3×10^9L, neutrophils segment — 82%, neutrophils bands — 3%; lymphocytes — 15%; hemoglobin — 52 g/L; hematocrit — 16%; platelets — 8000/μL; biochemical analysis: blood urea nitrogen — 22.7 mmol/L (2.0 to 7.0 mmol/L), creatinine —119 μmol/L (44 to

97 μmol/L in women and from 44 to 115 μmol/L in men); PT and aPTT within normal range; LDH — 6700 U/l (from 13 to 220 U/l. In women, 130—235 U/l. In men).

Question 1: What do these data show?

Answer: The patient has a febrile state, is slightly ailing, has anemia, thrombocytopenia and moderate azotemia. All this is consistent with a diagnosis of thrombotic thrombocytopenic purpura (TTP). As there is an effective specific therapy and the time factor is extremely important, the diagnosis must first be confirmed.

Question 2: What should be done to make the diagnosis?

Answer: Evaluate peripheral blood smear. White blood cell count — no abnormalities. Platelet count is reduced, they themselves are slightly elevated. Significant changes in the red blood cells. Many cells are helmet-shaped, triangular, smaller fragments can be seen. Similar schistocytes are characteristic of microangiopathic hemolytic anemia.

Question 3: What is the differential diagnosis of microangiopathic hemolytic anemia?

Answer: In addition to TTP (or hemolytic-uremic syndrome (HUS)), the following must be considered

a) Pathology of the heart and large blood vessels that can interrupt blood flow and cause cell destruction by mechanical injury. An example is «Waring blender» syndrome, in which red blood cells are compressed (flattened) by poorly functioning heart valve prostheses;

b) Anomalies of the microvessels, for example their congenital anastomoses;

c) immune lesions of the small vessels in acute glomerulonephritis, hypertension and malignant diseases.

They may be accompanied by local intravascular coagulation disorders and procoagulant disorders of the humoral coagulation system;

d) other microangiopathic processes (placental abruption, promyelocytic leukemia, snakebite);

e) inflammatory processes: Sepsis, pancreatitis, overheating, post-transfusion reactions.

A hyaline thrombus (consisting of aggregates of platelets cross-linked with fibrinogen and von Willebrand factor (vWF)) in terminal arterioles or capillaries can be tested for diagnosis. Bone marrow or gum biopsies help to detect similar changes in the vessels.

Considering the patient's medical history and examination data, the presence of thrombocytopenia and anemia, and normal levels of PT and aPTT, the most likely diagnosis is TTP. To maximize the effect, treatment should be initiated immediately.

Question 4: What should be done?

Answer: The treatment of choice is plasmapheresis with infusion of fresh frozen plasma (plasma exchange). Some authors believe that the development of TTP is related to the entry of unusual forms of vWF into the bloodstream when endothelial cells are damaged. These multimers bind more effectively than other forms of vWF to glycoprotein complexes on the surface of platelets. This binding leads to increased platelet aggregation and mechanical damage to the red blood cells as the aggregates pass through the system. Fresh frozen plasma provides an enzyme, apparently terminal disulfide reductase, which helps to rid itself of giant vWF complexes secreted by the affected endothelial cells (Fig. 2).

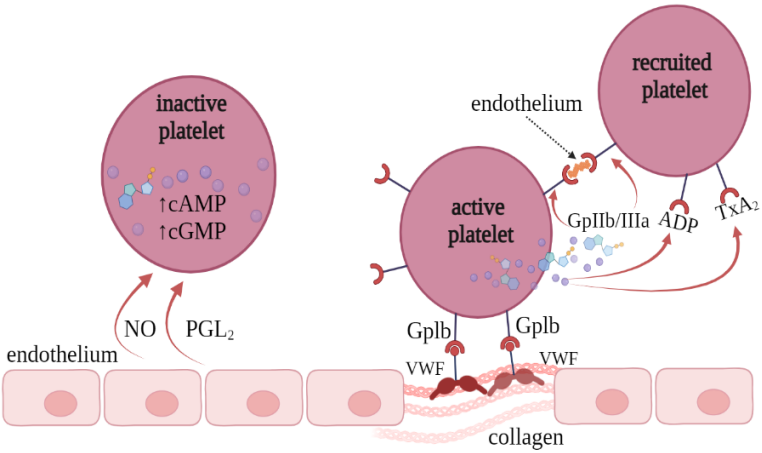

Fig. 2. Pathogenesis of thrombocytopenic purpura

Result. Intensive therapy helped the patient. Plasmapheresis and infusion of fresh-frozen, platelet-poor donor plasma (3—4 L/day) eliminated the haemolytic anemia and thrombocytopenia, lowered LDH levels, normalized renal function and eliminated the neurological changes.

Clinical Example № 5.

A 31-year-old woman with no history of pathology (excluding joint pain) went to the emergency room. She complained of red patches on her ankles and «bloody blisters» in her mouth. Menstruation started 1 week earlier than planned. The examination revealed hemorrhagic blisters on the oral mucosa, petechiae on the thighs and ankles. Liver, spleen and lymph nodes are not enlarged. Bleeding from the vagina. White blood cells — 11.2×10^9/L, hemoglobin — 117 g/L, hematocrit — 35%, platelets — 2.0×10^9/L.

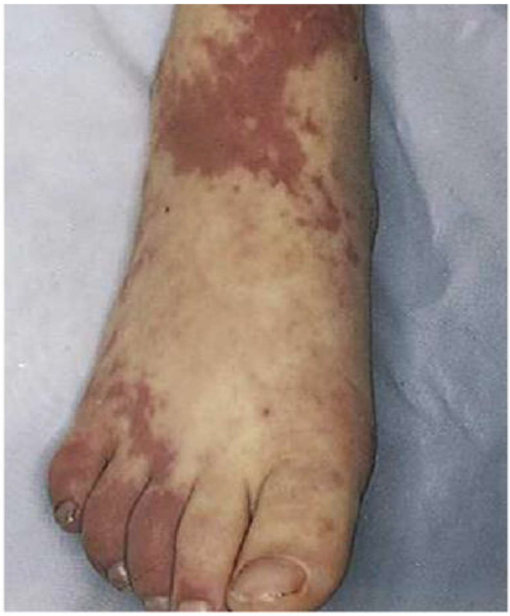

Fig. 3. Purpura

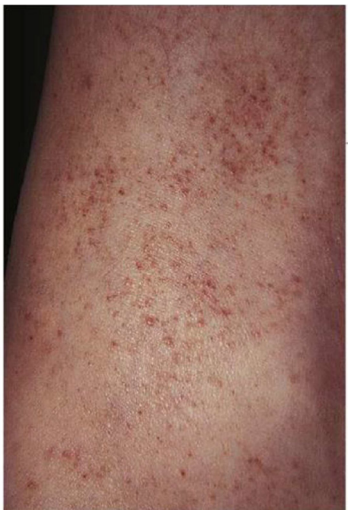

Fig. 4. Petechiae

Question 1: What should be done?

Answer. Examine the peripheral blood. Morphology of red blood cells and white blood cells unchanged. Platelets are small and large. Obviously accelerated platelet formation, many of which are immature but are actively consumed after entering the peripheral circulation.

Question 2: What should be done to make a diagnosis?

Answer. A bone marrow aspiration was performed. This revealed normal maturation of all hematopoietic sprouts, but many basophilic megakaryocytes. The level of platelet antibodies is not diagnostic. Since the patient shows signs of joint pathology, the rheumatoid process/collagenosis should be differentiated once the condition has stabilized.

Question 3: How should this patient be treated?

Answer. Since hemorrhagic vesicles (moist purpura) are seen on the oral mucosa and active uterine bleeding has been noted, treatment must be initiated immediately. Most clinicians prefer the use of high-dose prednisolone in oral form, but intravenous corticosteroids

and immunoglobulins can also be used. Although platelet transfusion does not always lead to a significant increase in the platelet count, it usually stops the bleeding quickly. There is no consensus among hematologists on the priority of these therapeutic approaches.

Question 4: The treatment has stopped the bleeding. The dose of corticosteroids was reduced, but after a few months the patient's platelet count dropped again to a dangerously low level. Despite two further attempts to maintain the platelet count at a sufficient level with a high dose of prednisolone and then slowly reduce it, a platelet counts of 1.0×10^9/L, increased hemorrhagic vesicles on the oral mucosa and minor gastrointestinal bleeding occurred. What therapeutic measures are required?

Answer. Splenectomy can be performed after prior immunization with vaccines against Streptococcus pneumoniae, Neisseria meningitidis, Meningococcus and Haemophilus influenzae; danazol or other immunosuppressive agents may be attempted or chemotherapy given, but these agents are usually left in reserve in case problems arise after splenectomy.

Outcome. The patient responded well to splenectomy, there was a sustained remission with an adequate platelet count.

Laboratory study of hemorrhagic diatheses

Laboratory examination of hemorrhagic diatheses. The laboratory investigation of bleeding disorders should include all components of the hemostatic mechanism: Vascular wall, platelets, clotting factors.

Laboratory screening tests for bleeding include

1. Determination of platelet count (count and measure).
2. Examination of the peripheral blood smear.
3. Bleeding time (BT) for suspected qualitative impairment of platelets or BT.
4. Activated partial thromboplastin time (APTT).
5. Prothrombin time (PV).

In the case of acquired violations, e. g., Disseminated intravascular coagulation (DIC), two additional samples are required:

6. Determination of fibrinogen content.
7. Determination of the content of fibrin degradation products (FDP) or D-dimers.

Examination of the blood smear provides further information about the cause of the abnormal platelet count. For example, if a low platelet count is found in the presence of fragmented red blood cells or schistocytes, this indicates increased platelet destruction as the reason for the low platelet count and the release of young large platelets from the bone marrow. The presence of myeloblasts at the same time as the low peripheral blood small platelet count indicates invasion of the bone marrow by leukemic cells causing a decrease in platelet production. Bleeding in a patient with a platelet defect may be due to a low platelet count (thrombocytopenia) (<100,000/μL), impaired platelet function with a normal platelet counts or qualitative disorders of these cells

With the *bleeding time (BT)* you can determine the state of the vessels during the interaction between blood platelets and the vessel wall. It is measured using the modified Ivy method. After the cuff has been applied to the upper part of the shoulder and a pressure of 40 mm Hg has been generated in it, an incision is made in the skin of the flexor side of the forearm (1×9 mm) using a disposable scalpel. BT is the time required to stop the bleeding, usually 3—8.5 minutes. The BT is normalized to the platelet count >100,000/μL. A lower platelet count is associated with a progressive increase in BT. The BT deviates from the norm in primary diseases of the vascular wall (e.g., vascular purpura) and in qualitative disorders of platelets and von Willebrand disease.

Activated partial thromboplastin time (APTT). It allows you to measure the internal factors of blood clotting (factors XII, PC, VMK, XI, IX, VIII) and common pathway factors (X, V, II, I). To conduct the sample, an activating agent (crushed silicon oxide or kaolin) is used — a substitute for platelet membrane phospholipids, calcium and the patient's citrate plasma or normal plasma. After the addition of the activating agent to the plasma, the active serine center of factor XII is «opened», which leads to the subsequent activation of both the coagulation factors of the internal pathway and the general pathway factors. The activator replaces platelet membrane phospholipid, which binds activated factors IX, X, V, and II to accelerate clot formation in the presence of added calcium. The end of the convolution is recorded in seconds. The value of APTT is normally 25—38 s. In case of a deficiency of blood coagulation factors XII, PC, BMC, XI, IX and VIII, as well as X, V, II and I APTT increases.

Activated partial thromboplastin time (APTT). It can be used to measure the internal factors of blood clotting (factors XII; plasma prekallikrein (PK), also called «Fletcher factor»; Fitzgerald factor (high molecular weight kininogen (HMWK or HK)); XI; IX; VIII) and the factors of the common pathway (X, V, II, I). To perform the test, an activator (crushed silica or kaolin) is used — a substitute for platelet membrane phospholipids, calcium and the patient's citrated plasma or normal plasma. The addition of the activator to the plasma "opens" the active serine center of factor XII leading to the subsequent activation of both internal and common pathway clotting factors. The activator replaces the phospholipid of the platelet membrane, which binds the activated factors IX, X, V and II and accelerates clot formation in the presence of added calcium. The end of folding is recorded in seconds. The value of the APTT is normally 25—38 seconds. If there is a deficiency in the blood coagulation factors XII, PK, Fitzgerald factor, XI, IX and VIII as well as X, V, II and I, the APTT increases.

Prothrombin time (PT). This test can be used to determine factor VII (external coagulation pathway) and factors X, V, II and I (common pathway factors). Tissue factor (TF) and calcium are added to the patient's plasma. The TF activates factor VII, which in turn activates the factors of the common pathway (X, V, $Ca2^{+}$ and factor II), which leads to the formation of thrombin. Thrombin converts fibrinogen into fibrin. PT does not take into account the state of the factors in the internal clotting pathway.

The normal range is 10—14 seconds. The PT can be expressed as an international normalized ratio (INR), which is calculated as follows: INR=(patient's PT of in seconds/PT of standard healthy human plasma) ^{ISI}, where ISI is an international sensitivity index that relates the activity of tissue factors from animal sources to the standard tissue factor in humans. INR values are used to monitor the effect of oral anticoagulants. The use of INR is recommended by the World Health Organization to achieve more accurate monitoring of anticoagulant treatment and to ensure comparability of data between laboratories.

The PT is increased in people with a hereditary deficiency of factors VII, X, V and I or an acquired combined deficiency of factors (vitamin K deficiency or oral intake of anticoagulants).

Interpretation of laboratory tests

Increased APTT. Screening and clotting tests performed before surgery can detect an increase in APTT. In the future, it is recommended to examine platelets, PT and BT (after a thorough history and general examination of the patient). In the laboratory examination of a patient with prolonged APTT, normal platelet count, normal prothrombin time and bleeding time, several tests must be performed in succession. Particularly important is the clinical determination of the patient's hemorrhagic status (whether he is bleeding or not). If the patient has sought medical attention for the first time because of direct bleeding, the platelet count, APTT, PT and BT must be determined after the medical history and physical examination.

In the case of bleeding and prolonged APTT, normal platelet count, normal PT and BT, the test for measuring APTT is performed in combined plasma: 1 volume of normal plasma +1 volume of patient plasma — the so-called 1:1 mixed APTT test. If a prolonged APTT is corrected in this test variant, a deficiency of factors VIII, IX and XI can be suspected, as only 30—50% of their normal value is required for a normal APTT. If, on the other hand, there is no correction, the presence of an inhibitor is suspected. In most cases, these are inhibitors that neutralize the activity of factors VIII or IX, but the presence of an inhibitor of factor XI activity is also suspected. In cases of bleeding and prolonged APTT, normal PT and platelet count but prolonged BT, the causes influencing the state of the internal circulation must be investigated and the state of platelet function checked.

Without bleeding with prolonged APTT with normal platelet count and APTT correction in the 1:1 test, one of the contact phase coagulation factors (e.g., factors XII, PK or HMWK) is deficient. Without APTT correction in the 1:1 test, these patients probably have a lupus-like inhibitor in their plasma, which is associated with thrombosis without hemorrhagic complications.

Increase in PT. Bleeding with prolonged PV (normal APTT, platelet count and VT) may indicate factor VII.

Increased APTT and PT. If bleeding occurs with prolonged APTT and PT (normal platelet count and BT) and is corrected in a 1:1 mixed test, the following conditions are likely:

1) deficiency of factors X, V, II or I (common pathway factors);
2) Vitamin K deficiency (insufficiency of all vitamin K-dependent factors: X, IX, VII and II);
3) Effects of warfarin therapy (also deficiency of vitamin K-dependent factors);
4) Liver disease (reduced synthesis of coagulation factors);
5) disseminated intravascular coagulation (see below);
6) active fibrinolysis (as a secondary event following therapy or a pathological process).

If bleeding occurs with prolonged APTT and PT (normal platelets and normal BT) but without correction in a 1:1 mixed test, it can be assumed that one of the common pathway factors is an inhibitor. In most cases, this is a factor V inhibitor, but factor X or factor II inhibitors are also possible.

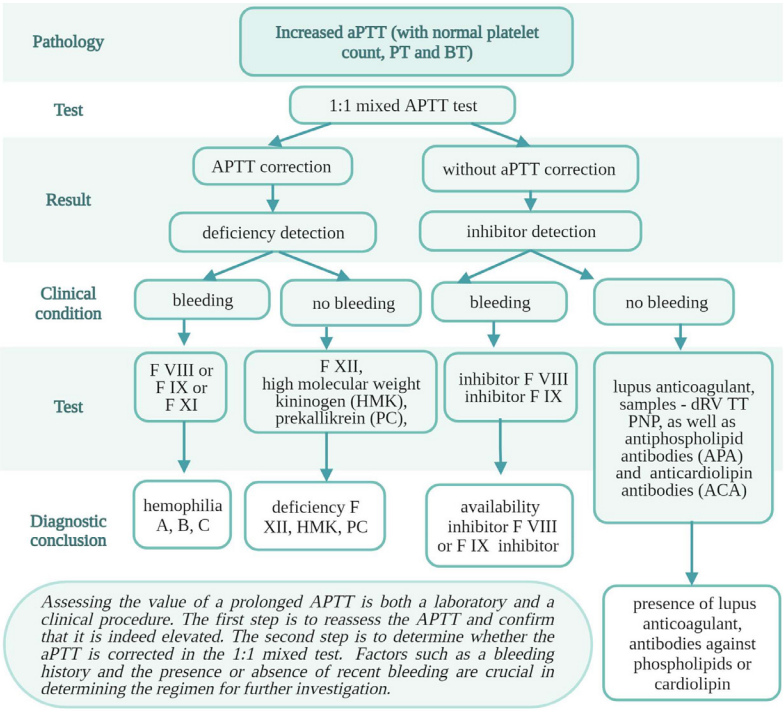

Fig. 5. Algorithm for diagnosing coagulopathies

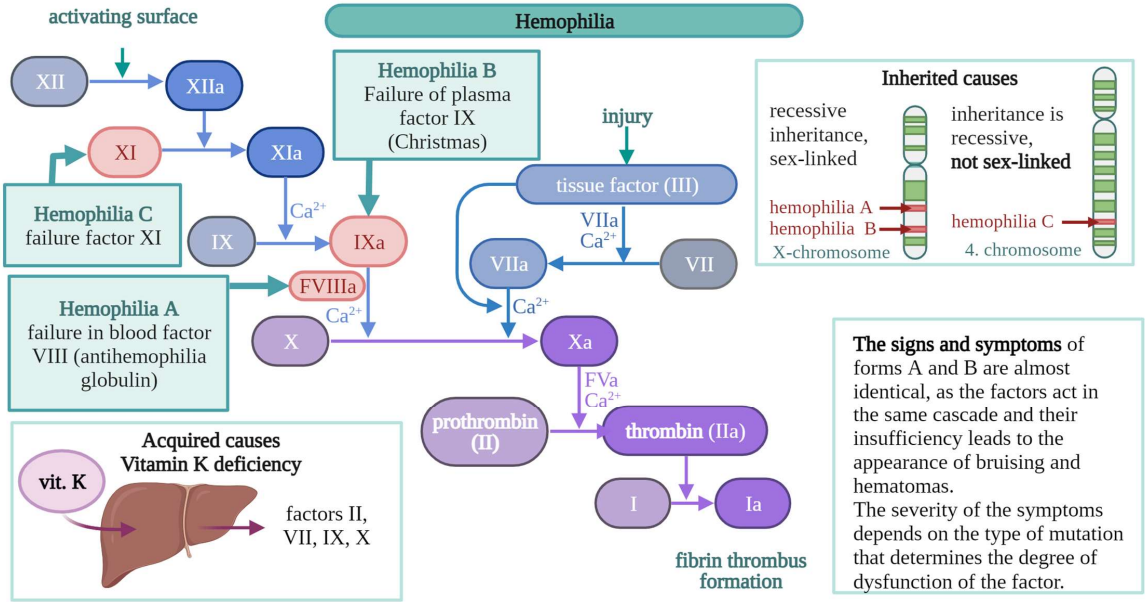

Fig. 6. Hemophilia

Clinical example №6.

The 22-year-old woman complains of the frequent appearance of bruises. A month ago, she had several bruises on her hips and shoulders without having suffered even minor trauma. According to the patient, there were problems with her periods: «It's much worse than with people I know» — longer and plentiful. There were nosebleeds in childhood, the bruising was slight. I had never had an operation, not even a tooth extraction. Pregnancy was not one of them. Her matter of younger sister so easily had bruises and bleedings; the father is healthy. The patient took no any medicine and trained as a nurse anesthetist. She did not abuse alcohol or drugs. There were no risk factors for HIV infection. At physical inspection of pathology of internals, it is not revealed. On the skin of the outer thighs and shoulders, ecchymoses in various stages of resolution with a diameter of 2—3 cm. On the mucous membranes there is neither petechiae, nor purples. Anomalies of the head, ears, eyes, nose, throat, neck, lungs and heart are not detected. The abdominal organs are unremarkable. Results of inspection of chest glands, rectum and genitals are normal. The neurological examination revealed no pathological findings.

Question 1: What disease can be assumed?

Answer. The most characteristic hereditary disease associated with bleeding is von Willebrand disease (about 1 in 1000 people). The frequency of hemophilia and is 1 in 10,000 men (inheritance of a defective X chromosome). The development of symptomatic coagulopathy in women is only possible if two abnormal X chromosomes are inherited. This is certainly the case with a healthy father. However, if symptoms occur in the mother and sister, the most likely genetic defect in coagulation in this woman is von Willebrand disease, but not other inherited defects. It is possible that this is an acquired atrial fibrillation disorder, especially given the recent increase in symptoms. In any case, the family history must be carefully investigated.

Question 2: What should be done first?

Answer. The laboratory examination must begin with the determination of PT and APTT to assess the humoral system of blood clotting. A peripheral blood smear needs to be analyzed to determine platelet count, and then platelet function may need to be assessed —

to determine bleeding time. Abnormalities in ciliated factors usually lead to the occurrence of ecchymoses and other hemorrhagic injuries. Qualitative and quantitative platelet injuries lead to the appearance of petechias and purple, and in some cases — ecchymosis.

Question 3: It turned out that PT — 11 with; APTT — 34 with (i.e., both indicators are normal). Platelet count — 345×10^9/L with normal peripheral blood smear. BT — 8 min (normal). What to do next? Is it possible to tell the patient that she is healthy?

Answer. Although the laboratory values show no pathological changes, APTT and BT are at the upper limit of the norm. In von Willebrand disease, the size of the indicators may be normal or may deviate slightly from the norm at different times. Hormonal or other factors leading to the development of clinical or laboratory abnormalities may be involved in the control of the humoral system of blood coagulation (the woman must be examined in the first or second week of her menstrual cycle). Therefore, the determination of the indicators should be repeated the next month.

Question 4: After the repeated examination APTT — 39 with; VT — 9.5 min. what to do next?

Answer. To watch for the increased APTT, 1:1 micro test should be performed (patient plasma + normal plasma). This corrects for the absence of a factor when there is no inhibitor in the patient's plasma. The patient's APTT actually decreased to 27 with. Given the elevated BT and APTT, it is possible to make the diagnosis of von Willebrand's disease (folding system deficiency plus platelet defect) and perform screening tests for von Willebrand's disease. In addition, the following results are obtained: (factor VIII: C — 50% (norm of 50—180%); the cofactor of a ristocetin Cofactor — 50% (norm of 50—150%); antigen of a factor of von Willebrand — 50% (norm of 50 — 180%).

Question 5: Is this the final diagnosis? Is it necessary to do something else?

Answer. The examination results are fully consistent with von Willebrand disease, but it is necessary to determine the subtype, as treatment of the desmopressin lineage in patients with type PT can lead to platelet consumption and thus to a worsening of the coagulation condition. In patients with type I, the most common type, such complications do not occur with desmopressin treatment.

Question 6: How can a subtype be defined?

Response. The Western blot method was used to test for von Willebrand multimeter. In this patient, the multimeters were qualitatively normal, but their quantity is so reduced that type I BT allows the diagnosis. Now it is necessary to give all the information about the patient.

Observation. 1 year after the diagnosis, the wisdom tooth was successfully extracted with preventive intake of desmopressin. The patient feels well.

Clinical Example № 7.

A 23-year-old woman came to the emergency room because of swelling and pain in her left calf. She was previously healthy and had no serious medical conditions that required hospitalization. She was taking daily contraceptives. Three days before the treatment, she felt discomfort in the left calf area, which was painful, swollen and reddened. There was also pain in her hip. The patient has never been pregnant, does not smoke, does not drink alcohol and does not take painkillers. No risk factors for HIV infection were identified. He works as an employee. No limbs have been immobilized recently and the patient has not taken a vacation. Her uncle died at the age of 37 after undergoing surgery. The examination revealed swelling, redness, hyperthermia, left caviar is painful. No venous cords were found, but there are signs of edema. The lymph nodes in the groin area are not enlarged, painless and soft. The laboratory values are normal. Ultrasound examination revealed deep vein thrombosis in the groin and hip. Heparin therapy was prescribed and after 24 hours — warfarin.

Question 1: Is it necessary to examine the patient for a hypercoagulable state?

Answer. Yes. A family history and apparently unprovoked deep vein thrombosis may be signs of hereditary hypercoagulability triggered by contraceptive use.

Question 2: What investigations should be carried out?

Answer. Mass screening for predisposition to hypercoagulation of expensive but very parsimonious tests is currently performed in

many laboratories to identify the most common abnormalities that cause the hypercoagulable state. Testing for antithrombin III, protein C, protein S deficiency, elevated levels of lupus anticoagulant (LA) and other antiphospholipid antibodies, resistance to activated protein C, and elevated homocysteine.

Question 3: When is the best time to do it?

Answer. The blood sample must be taken before starting treatment with heparin or warfarin, as heparin alters antithrombin III levels and warfarin — protein S and protein C. Due to the active formation of the clot, a number of tests may give false results. It is therefore necessary to discontinue the therapy and carry out the tests 2 weeks later. Functional tests in the screening test for activated protein C showed a low level of this protein. As a result, a mutation of factor V Leiden was detected.

Question 4: What should the patient be advised to do?

Answer. The patient has a predisposition to increased blood clotting due to the disorder detected. It is undesirable for her to take contraceptives containing estrogen, as these cause a state of hypercoagulation. In addition, prophylaxis with anticoagulants is necessary if limbs are immobilized, e.g., during surgical procedures. Whether the patient should take anticoagulants for the rest of her life if she has no other risk factors has not yet been clarified. Anticoagulant therapy may be necessary if estrogen levels are high, e.g., during pregnancy. It is advisable to carry out screening tests on her relatives so that they can also be given recommendations.

Observation. The patient informed her relatives about her condition and convinced them to get tested. After a discussion with her doctor, she decided to stop taking contraceptives but did not agree to be treated with anticoagulants and decided to come back to this issue in case of pregnancy.

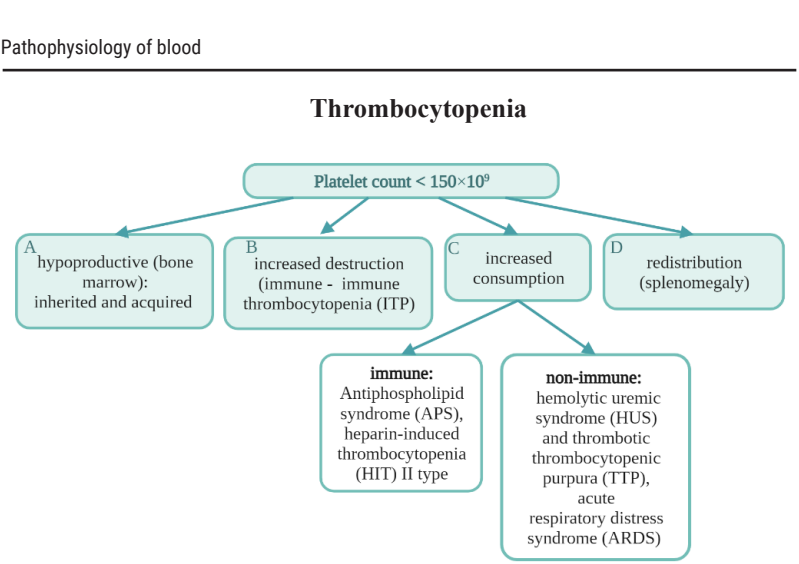

Fig. 7. Classification of thromocytopenia

Table 3

Algorithm for the diagnosis of thrombocytopenia A and B

Indicators	Hypoproductive	Immune
Reticular platelets	↓	Norm or ↑
Glycocalycin	↓	Normal, ↑, or ↓ (if at inhibit thrombocytes Poez)
Thrombopoietin	↑Free thrombopoietin, ↓Bound thrombopoietin	Norm
TA-IgG	Norm, ↑ (after blood transfusion)	↑ (40—60%) If TA-IgG is not elevated, it means that it is bound to platelets and undetectable in plasma

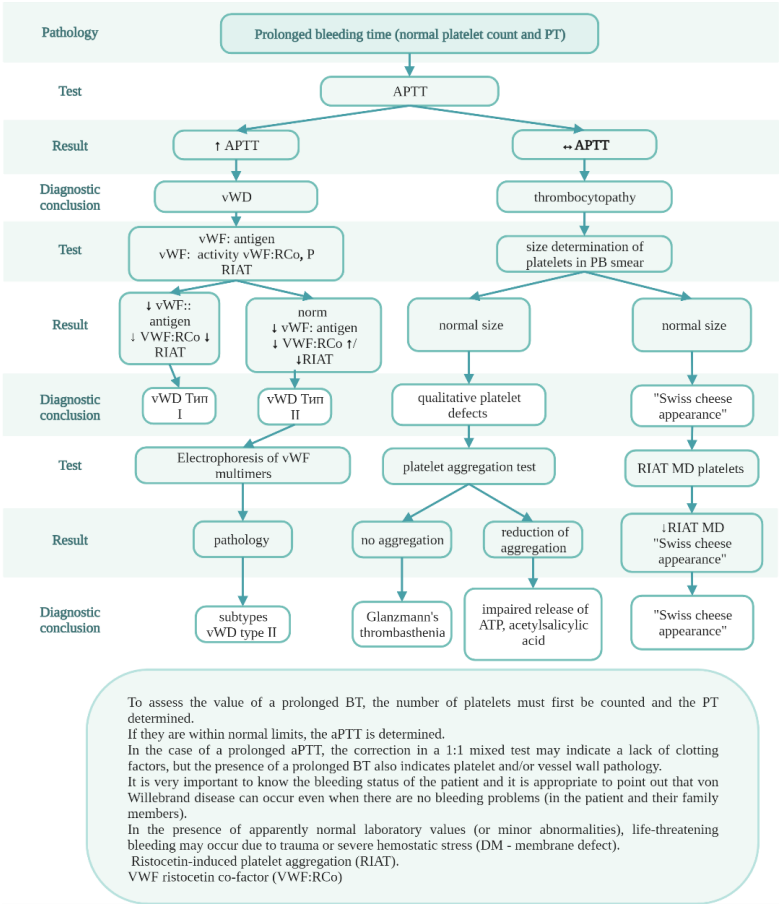

Fig. 8. Algorithm for the diagnosis of thrombocytopenia

Determination of platelet-associated immunoglobulins (TA-IgG)

Determination of reticular («young» forms) platelets.

Reticular, «young» forms of platelets contain a larger amount of ribonucleic acid (RNA) and therefore they are detected by col-

oration with RNA-specific fluorescent dyes (thiazole orange, etc.) using flow cytofluorimetry. The percentage of reticular platelets is significantly increased in ITP patients, both compared to healthy donors and patients with hypoproductive thrombocytopenia. This is explained by the accelerated production of platelets, which is observed in many patients with ITP, especially during the acute period of the disease (in contrast to patients with hypoproductive thrombocytopenia). However, in some ITP patients, mainly in the case of chronic disease, reticular platelets are not necessarily increased, due to the fact that in such patient's partial inhibition of thrombocytopoiesis may occur — presumably due to the effect of autoantibodies on bone marrow megakaryocytes.

Thrombocytopenia of increased destruction (Idiopathic thrombocytopenic purpura (ITP)):

— **ITB** of childhood against the background of viral infection (hapten)

— **alloimmune**: mother-fetus; donor recipient

The clinical manifestations of ITP are bleeding:

• dry purpura (on the legs);

• nosebleeds, from the gums and the gastrointestinal tract;

• wet purpura (in the oral cavity);

• intracranial bleeding (the most dangerous).

Thrombocytopenia of consumption of a non-immune nature is much less common and develops due to the participation of a significant number of platelets in thrombus formation. A decrease in blood platelet count by this mechanism is observed in disseminated intravascular coagulation syndrome, thrombotic thrombocytopenic purpura and hemolytic uremic syndrome, massive bleeding and thrombosis, and rare forms of hereditary pathologies associated with an increased tendency of platelets to intravascular aggregation.

Clinic:

• thrombocytopenia;

• microangiopathic hemolytic anemia due to red blood cell fragmentation;

• ischemia;

• thrombotic thrombocytopenic purpura (TTP) — neurological symptoms, hemolytic-uremic syndrome (HUS) — renal dysfunction.

The main link in the pathogenesis of HUS: endothelial damage and increased vWF formation. Occlusive blood clots consist of EF, thrombin, a small amount of fibrinogen or fibrin.

The main link in the pathogenesis of TTP: ADAMATS-13 deficiency → impaired vWF degradation. Epidemiology: 3.7 per 1,000,000. Fatal outcome 10—20%.

Thrombocytopenia of immune nature consumption. Platelet uptake as a result of intravascular thrombosis may also contribute to thrombocytopenia in antiphospholipid syndrome and rare forms of immune thrombocytopenia associated with platelet activation. The best-known example is heparin-induced thrombocytopenia (HIT) type II.

Heparin-induced thrombocytopenia.

HIT is the most frequently reported drug-induced form of thrombocytopenia. It occurs after the administration of unfractionated heparin and much less frequently with the use of low molecular weight heparins. There are type I and type II HITs.

Type I HIT is not immune in nature and is due to the insignificant activation of platelets by heparin and the potentiating effect of heparin on platelet activation by agonists such as adenosine diphosphate (ADP), collagen, etc. This type of HIT is quite common and is characterized by a moderate decrease in platelet count shortly (in the first few days) after the introduction of heparin and a rapid recovery after its discontinuation.

Type II HIT is immunologically caused and results from the formation of hapten antibodies against the heparin-TF4 complex. The antibodies in the composition of such complexes, which are fixed on the surface of platelets, interact with the platelet Fc receptor and stimulate its activation, leading to the formation of platelet aggregates and intravascular thrombi. The inclusion of platelets in such a thrombus leads to a significant decrease in the circulating platelet concentration — usually by more than 50% of the initial value and often below 50×10^9/L. The HIT type II clinic is a deep thrombocytopenia associated with thrombosis. Another

clinical sign characteristic of HIT type II is the manifestation of the disease 5—10 days after starting heparin. HIT type II occurs in about 1—3 % of cases with prolonged use of unfractionated heparin, most commonly in orthopedic and cardiovascular surgery after prolonged heparin therapy. Venous thromboses predominate, especially at sites with vascular injuries caused by catheters. Atypical manifestations such as bilateral adrenal hemorrhage, venous gangrene of the extremities and skin necrosis should be a reason for diagnostic consideration of GIT heparin-induced thrombocytopenia.

Laboratory diagnosis of thrombocytopenia of immune genesis consumption (type II HIT):

1. autoAT to the complex: «heparin-TF4»

The main link of the HIT pathogenesis: heparin activates platelets through ADP, collagen → thrombosis and deep thrombocytopenia ($<50\times10^9$/L).

Laboratory diagnosis of thrombocytopenia of non-immune origin Consumption:

1. Thrombocytes less than 20×10^9/L
2. Schistocytes in the smear
3. Increase in Ret
4. Indirect bilirubin increased
5. LDH increase (source-destroyed red blood cells and ischymized tissue)
6. APTT, PT, fibrinogen — normal
7. Creatinine increased (HUS (hemolytic-uremic syndrome) > TTP)

Table 4

Laboratory results

Index	TTP	DVS
AT III	norm	reduction

Treatment of thrombocytopenia of non-immune origin consumption — plasmapheresis.

List of recommended references:

1. *Momot A.P.* The problem of thrombophilia in clinical practice // Russian Journal of Pediatric Hematology and Oncology (RZHDiO). 2015. Vol. 2, №1. P. 36—48.

2. *Kienast J., Schmitz G.* Flow cytometric analysis of thiazole orange uptake by platelets: a diagnostic aid in the evaluation of thrombocytopenic disorders // Blood. 1990. Vol. 75. P. 116—121.

3. *Kurata Y., Hayashi S., Kiyoi T. et al*. Diagnostic value of tests for reticulated platelets, plasma glycocalicin, and thrombopoietin for discriminating between hyperdestructive and hypoplastic thrombocytopenia // Am J Clin Pathol. 2001. Vol. 115. P. 656—664.

4. *Pruthi R.K., Daniels P.R., Nambudiri G.S. et al.* Heparin-induced thrombocytopenia (HIT) during postoperative warfarin thromboprophylaxis: a second example of postorthopedic surgery 'spontaneous' HIT // J Thromb Haemost. 2009. Vol. 7, №3. P. 499—501.

5. *Mazurov A.V., Haspekova S.G., Vasiliev S.A*. Diagnosis of thrombocytopenia // Therapeutic archive. 2018. Vol. 90, №7. P. 4—13.

6. *Schiffman F.D*. Blood Pathophysiology: Tutorial manual. St. Petersburg, 2001.

7. *Novitsky V.V., Urazova O.I.* Pathophysiology : textbook. Vol. 2. M., 2018.

8. *Novitsky V.V., Goldberg E.D., Urazova O.I.* Pathophysiology : textbook. Vol. 1. M., 2015.

9. *Kienast J., Schmitz G.* Flow cytometric analysis of thiazole orange uptake by platelets: a diagnostic aid in the evaluation of thrombocytopenic disorders // Blood. 1990. Vol. 75. P. 116—121.

10. *Kurata Y., Hayashi S., Kiyoi T. et al.* Diagnostic value of tests for reticulated platelets, plasma glycocalicin, and thrombopoietin for discriminating between hyperdestructive and hypoplastic thrombocytopenia // Am J Clin Pathol. 2001. Vol. 115. P. 656—664.

11. *Mazurov A.V*. Physiology and pathology of platelets. M., 2011.

12. *Michelson A.D*. The clinical approach to disorders of platelet number and function // Platelets (Third Edition). 2013. Ch. 39. P. 813—818.

13. *Greinacher A., Warkentin T.E., Chong B.H.* Heparin — induced thrombocytopenia // Platelets. Elsevier, 2013. P. 851—882.

14. *Hong A.P., Cook D.J., Sigouin C.S. et al.* Central venous catheters and upper-extremity deep-vein thrombosis complicating immune heparin-induced thrombocytopenia // Blood. 2003. Vol. 101. P. 3049—3051.

15. *Ernest D., Fisher M.M.* Heparin-induced thrombocytopaenia complicated by bilateral adrenal haemorrhage. Intensive Care Med. 1991. Vol. 17. P. 238—240.

16. *Warkentin T.E., Roberts R.S., Hirsh J. et al.* Heparin-induced skin lesions and other unusual sequelae of the heparin-induced thrombocytopenia syndrome: a nested cohort study. Chest. 2005. Vol. 127. P. 1857—1861.