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ПАТОФИЗИОЛОГИЯ КРОВИ

Учебно-методическое пособие
для самостоятельной работы студентов 3 курса
факультета по подготовке студентов для зарубежных стран,
обучающихся на английском языке по специальности
«Лечебное дело», медицинских вузов

PATHOPHYSIOLOGY OF BLOOD

Teaching workbook
for self-training students for 3rd year students
of the Faculty on preparation of experts for foreign countries,
studying in english on specialty «General medicine»
of medical higher educational institutions

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В учебно-методическом пособии содержатся сведения об этиологии, патогенезе, классификации, диагностике и принципах терапии патологии системы крови в соответствии с типовой учебной программой по специальности «Лечебное дело».

Предназначено для самлстоятельной работы студентов 3 курса факультета по подготовке студентов для зарубежных стран, обучающихся на английском языке медицинских вузов.

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THE LIST OF ABBREVIATIONS

AA	— aplastic anemia
ADP	— adenosine diphosphate
AHG	— antihemophilic globulin
AIHA	— autoimmune hemolytic anemia
ALL	— acute lymphocytic leukemia
AML	— acute myelogenous leukemia
AP	— acid phosphatase
BAS	— biological active substances
BFU-E	— erythroid bursts forming units
BM	— bone marrow
CBV	— circulating blood volume
CD	— cluster differentiation
CFU-Ba	— basophilic colony-forming unit
CFU-E	— erythrocyte colony-forming unit
CFU-Eo	— eosinophilic colony-forming unit
CFU-G	— granulocyte colony-forming unit
CFU-GEMM	— colony-forming unit granulocyte-erythrocyte-monocyte-megakaryocyte
CFU-GM	— colony-forming unit granulocyte-monocyte
CFU-M	— monocytic colony-forming unit
CFU-Mk	— megakaryocytic colony-forming unit
CFU-N	— neutrophilic colony-forming unit,
CLL	— chronic lymphocytic leukemia
CML	— chronic myelogenous leukemia
CMV	— cytomegalovirus
CPG	— coproporphyrinogens
CSF	— colony stimulating factor
CSF	— colony stimulating factors
DIC	— Disseminated intravascular coagulation
DNA	— deoxyribonucleic acid
EBV	— Epstein-Barr virus
ESR	— Erythrocyte Sedimentation Rate
FAB	— French-American-British
FSF	— fibrin stabilizing factor
G-6-PD	— glucose-6-phosphate dehydrogenase
HA	— hemolytic anemia
Hb	— hemoglobin
HCD	— Heavy chain disease
HDN	— hemolytic disease of newborn

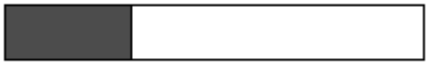
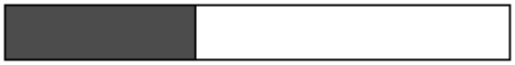
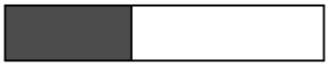
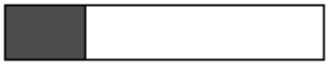
HIV — human immunodeficiency virus
HL — Hodgkin lymphoma
HMWK — high molecular weight kininogen
Ht — Hematocrit
HTLV-1 — human T-cell leukemia virus-1
IDA — iron deficiency anemia
IF — interferon
Ig — immunoglobulin
IL — interleukin
KSHV/HHV-8 — Kaposi sarcoma herpesvirus/human herpesvirus-8
LDH — lactate dehydrogenase
MM — Multiple myeloma
MPO — Myeloperoxidase
NADPH — nicotinamide adenine dinucleotide phosphate
NHL — Non-Hodgkin lymphoma
NK cells — natural killer cells
NSE — nonspecific esterase
NSI — nuclear shift index
PAF — Platelet-activating factor
PAS — periodic acid Schiff stain
PF — platelet factor
Pg — prostaglandins
PPK — plasma prekallikrein
PTA — Plasma thromboplastin antecedent
PTC — plasma thromboplastin component
RBC — red blood cell
RES — reticuloendothelial system
Rh — rhesus
SAA — sideroahrestical anemia
SPCA — serum prothrombin conversion accelerator
TIBC — total iron-binding capacity
TNF — tumor necrosis factor
TxA2 — thromboxane A2
vWF — Von Willebrand factor
WBC — white blood cells
WHO — World Health Organization

CHAPTER 1
BLOOD VOLUME AND HEMATOCRIT DISORDERS

The total blood volume taken to calculate body weight (approximately 6–8 %), so that an adult male blood volume is approximately 5 liters. In this case 3.5–4 liter normally circulates in bloodstream and heart cavities (the circulating fraction of blood), and 1.5–2 liters deposited in the vessels of the abdominal cavity, lungs, subcutaneous tissue and other tissues (deposited fraction). Forming elements are up 36–48 % of total blood volume.

Hematocrit (Ht) — is the ratio of blood cells to plasma volume. Normally equal to 0,41–0,50 for men, women 0,36–0,44).

Table 1 — Typical forms of changes in the total volume and / or the ratio of the formed elements and blood plasma

Total blood volume (formed elements/plasma)	**Condition of blood volume**
	Norm
	Oligocythemic normovolemia
	Polycythemic normovolemia
	Normocythemic(simple) hypervolemia
	Oligocythemic hypervolemia
	Polycythemic hypervolemia
	Normocythemic(simple) hypovolemia
	Oligocythemic hypovolemia
	Polycythemic hypovolemia

NORMOVOLEMIA

Normovolemia — is a condition, that characterized by normal total blood volume, combined with a decreased, an increased or a normal Ht. There are oligocythemic, polycythemic and normocythemic normovolemia. Normocythemic normovolemia is normal state of blood.

Oligocythemic normovolemia

Oligocythemic normovolemia — is a condition with a normal total blood volume with a decreased number of formed elements (mostly red blood cells), accompanied by a fall in hematocrit values below normal (< 36 %).

The main reasons are:

- massive hemolysis (eg, formation of antierythrocytic Ig; hemolytic action of substances — snake venom, lead compounds, arsenic, phenylhydrazine, etc.);
- prolonged and extreme inhibition of hematopoiesis, mainly of erythropoiesis (eg, aplastic anemia);
- states after blood loss, acute bleeding (in this case, the total blood volume become normal relatively quickly due to transport of fluid from the tissues into the bloodstream, and the number of blood cells is still reduced).

Manifestations:

- anemia (due to the decrease the number of red blood cells) and as a consequence — hemic hypoxia;
- thrombocytopenia (blood loss, or immune reactions autoaggression for platelets);
- reduction of blood coagulability, often combined with a hemorrhagic syndrome;
- leukopenia, which determines the reduction of anticontagious resistance of the organism;
- reduction of blood viscosity. (eg, during hydremic compensate for acute blood loss).

Polycythemic normovolemia

Polycythemic normovolemia — is a condition characterized by normal total blood volume and increasing the number of formed elements, which is accompanied by an increase Ht above normal (> 48 %).

The main reasons are:

- chronic hypoxia (erythrocytosis is due to the activation of erythropoiesis);
- infusions to patients fractions of blood cells (erythrocytes, leukocytes or platelets);
- erythremia.

Manifestations:

- increase of blood viscosity;
- thrombotic syndrome;
- microcirculation disorders (slowing of blood flow in microvasculature, stasis), which cause reduction of transcapillary exchange in the tissues;
- hypertension (by increasing cardiac output).

HYPERVOLEMIA

Hypervolemia — is a conditions characterized by an increase in total blood volume, and usually changing Ht. There are normocythemic, oligocythemic, polycythemic hypervolemias.

Normocythemic hypervolemia

Normocythemic hypervolemia (simple) — is a condition manifested by an equivalent increase in the amount of formed elements and the liquid part of the CBV. Ht is at the range of normal.

The main reasons are:

- a large amount of blood transfusions;
- acute hypoxic state;
- accompanied by ejection the blood from pool;
- heavy physical activity, leading to hypoxia.

Manifestations:

- circulatory disorders due to hyperextension of vessels and heart cavities and microcirculatory disorders.

Oligocythemic hypervolemia

Oligocythemic hypervolemia (hydremia, hemodilution) — is a condition characterized by an increase in total blood volume due to the increase of its liquid part. Ht is lower than normal (< 36 %).

The main reasons are:

- excessive intake of fluid in pathological thirst (for example, patients with diabetes melitus) and transfusion a large number of plasma substitutes or blood plasma;
- reduction of removing fluid from the body as a result of failure of excretory renal function (eg, renal failure), overproduction of ADH, hyperosmolality of blood plasma.

Manifestations:

- circulatory disorders due to hyperextension of vessels and heart cavities and microcirculatory disorders.

Polycythemic hypervolemia

Polycythemic hypervolemia — is a condition manifested by an increase in total blood volume due to the increase in the number of formed elements. Ht is upper limit of normal (> 48 %).

The main reasons are:

• erythrocytosis — a group of pathological conditions characterized by an increase in the number of erythrocytes (regardless of the number of white blood cells, platelets);

• polycythemia (polycythemia vera, Vaquez disease) — chronic leukemia with a lesion at the level of progenitor cells myelopoiesis with unlimited proliferation and ability to differentiate mainly in the RBCs as a result is an increased Ht;

• chronic hypoxia of any types (hemic, respiratory, circulatory, tissue, etc.).

Manifestations:

• increased cardiac output (the result of compensatory hyperfunction of the heart due to increased blood volume; however, cardiac output is usually reduced in decompensation heart failure);

• increased blood pressure (due to the increase cardiac output, CBV and the tone of resistive vessels);

• increased blood viscosity;

• increased aggregation and agglutination of blood cells;

• disseminated thrombosis;

• disorders of microcirculation.

HYPOVOLEMIA

Hypovolemia — is a conditions characterized by a decrease in total blood volume and, as a rule, ratio distortion of the formed elements and plasma. There are normocythemic, oligocythemic, polycythemic hypovolemia.

Normocythemic hypovolemia

Normocythemic hypovolemia — is a condition manifested by a decrease in total blood volume, while maintaining Ht within normal limits.

The most common reasons:

• acute blood loss;

• shock, vasodilatory collapse.

In this are two cases normocythemic hypovolemia that caused by deposition of a large blood volume in the venous (capacitance) vessels and intense reduction of CBV.

Manifestations:

Determined by the nature of the reasons that caused it (blood loss, shock, collapse), and the starting compensatory mechanisms to the abatement acute hypoxia.

Oligocythemic hypovolemia

Oligocythemic hypovolemia — is a condition characterized by a decrease in total blood volume with a primary decrease in the number of formed elements. Ht is below normal (< 36 %).

The most common reasons:

- states after acute blood loss;
- erythropenias is a result of massive hemolysis of RBCs (for example, burns a large area of the body, when hemolysis is combined with the plasmorrhagia) and suppression of erythropoiesis (eg, aplastic or aregeneration states).

Manifestations:

- the decline in blood oxygen capacity (as a result erythropenia);
- signs of hypoxia (eg, reducing the oxygen content in blood, acidosis, a decrease of venous blood pO_2, etc.);
- disorders of blood circulation and microcirculation in organs and tissues varying degrees, due to a decrease in CBV.

Polycythemic hypovolemia

Polycythemic hypovolemia — is a condition with decreasing total blood, mainly due to a decrease in plasma volume. Ht is above the range of normal (> 48 %).

The most common reasons:

- states, causing an increased loss of body fluid: repeated vomiting (eg pregnancy or as a result of exogenous intoxication), prolonged diarrhea, polyuria (eg, renal failure), increased and prolonged sweating, and extensive burns of the skin (accompanied plasmorrhagia);
- conditions prevent adequate flow of fluid in the body: the lack of potable water and the impossibility of drinking water (eg due to muscle spasm in tetanus or rabies).

Manifestations:

- organs and tissues microcirculation disorders due to hypovolemia, and polycythemia;
- increase blood viscosity, aggregation of blood cells in microcirculation vessels of organs and tissues and disseminated microthrombosis;
- the signs of main pathologies causing polycythemic hypovolemia (eg, shock, diabetes insipidus, renal failure, burn patients, etc.).

BLOOD LOSS

Blood loss — is a pathological condition as a result of hemorrhage that lead to disturbances of vital activity of the organism in a various degree.

Etiological factors of blood loss:

1) violation of the blood vessels integrity (injury, damage by pathological processes);

2) increased permeability of the vascular wall;

3) decrease of blood clotting (haemorrhagic syndrome).

Pathogenesis of bleeding has three stages:

1) initial;

2) compensatory;

3) terminal.

1. **Initial stage.** Decreased CBV (hypovolemia), decreased cardiac output and blood pressure develop circulatory hypoxia.

2. **Compensatory**. Distinguish the following phases of compensatory reactions:

1) ***Vascular reflex phase*** lasts 8–12 hours from the start of hemorrhage. It is characterized by spasm of peripheral vessels due to release of adrenal catecholamines, which results leads to reduce the volume of bloodstream («centralization» of blood circulation) and helps maintain blood flow to vital organs (brain and heart). At the same time there is activation of the renin-angiotensin-aldosterone system that leads to activation the processes of sodium and water reabsorption in the proximal tubules of the kidneys, accompanied by a decrease in diuresis and water retention in the body. During this period, the loss of blood plasma and blood cells and as a result equivalent compensatory out of blood from pool, as a result the hematocrit about norm («hidden» anemia). Early signs of acute blood loss are leukopenia (in some cases, may be leukocytosis) and thrombocytopenia.

2) ***Hydremic phase*** (in 1–2 days after blood loss). On this stage there is restoration of plasma volume. Mobilize tissue fluids in to blood stream. «Dilution» of blood is accompanied by a progressive decrease in the number of RBCs and hemoglobin in a unit volume of blood. The anemia has normochromic, normocytic character.

3) ***Phase of bone marrow*** (develop into 4–5th day after the hemorrhage). Juxtaglomerular apparatus of kidneys is response to hypoxia by overproduction of erythropoietin, which stimulates activity of unipotent progenitor cells of erythropoiesis — CFU-E. In case of sufficient regenerative capacity of bone marrow there is increase of young forms of red blood cells (reticulocytes) in the blood (after 4–5 days). It is accompanied by changes in the size of red blood cells (macrocytosis) and cell shape (poikilocytosis). In the blood can appear RBCs with basophilic granules, sometimes single normoblasts, develops a mild leukocytosis (up to 12×10^9/l) with a left shift to metamyelocytes (rarely up to myelocytes) and increase in platelet counts (up to 500×10^9/l or more).

Protein compensation is realized by activation proteosynthesis in the liver, start from few hours after the bleeding, can be founded 1,5–3 weeks.

3. **Terminal stage** may occur in acute massive blood loss exceeding 50 % of CBV, absence of adequate treatment, insufficiency of adaptation (due to severe diseases, effect of unfavorable exogenous and endogenous factors).

In the case of acute blood loss (more than 15 % of CBV) significantly reduced venous return to the right heart, which leads to a decrease in cardiac output, a progressive drop in blood pressure and slowing down blood flow. In response to a decrease in central hemodynamics occurs systemic vasoconstriction, release deposited blood, and develops tachycardia and other compensatory mechanisms of hypovolemia. It allows maintaining blood pressure in the subcritical

level (90–85/45–40 mm Hg) until a certain time (until blood loss does not exceed 40–45 % of CBV). Continuing bleeding leads to the depletion of the body's adaptive systems (involved in the fight against hypovolemia), thus developed hemorrhagic shock. In this case the protective reflexes of macrocirculatory system are already insufficient to ensure adequate cardiac output, resulting in systolic blood pressure falls rapidly to critical numbers (50–40 mm Hg). Ultimately disturbed blood flow to organs and body systems, develops oxygen starvation, and death due to respiratory paralysis and cardiac arrest.

Acute Hemorrhage

Acute hemorrhage occurs after injury of a large vessel. In this case cellular elements and liquid part of the blood are lost proportionally (simple hypovolemia).

Acute loss of the blood up to 10 % of the blood volume and slow loss of even greater amounts may have no significant manifestations. Sudden loss of 25–40 % and more of the blood is dangerous for life. Loss of 60 % of blood is lethal.

A state of hemostasis system plays an important role; thus, in its disorder, a damage of even not so large vessel may lead to acute blood loss.

In clinical manifestations following changes play the critical role:

- acute disorder of the systemic blood circulation;
- critical decrease in arterial blood pressure;
- decrease in heart filling and systolic heart volume, coronary insufficiency (decrease of coronary blood supply);
- development of acute hypoxia of circulatory type;
- acute kidney insufficiency;
- acute posthemorrhagic anemia;
- hemorrhagic shock may occur if a compensation fails and is characterized by extreme disorder of all vital functions, loss of consciousness and death if not treated.

Chronic Hemorrhage

Causes of a chronic hemorrhage, as a rule, are of endogenous origin. They are bleeding from stomach or intestine ulcer, cancer of stomach or intestine, bronchial hemorrhage with pulmonary tuberculosis, massive menses in women, uterine hemorrhages. Chronic blood loss is accompanied by the development of the deficit of iron. In gastrointestinal bleeding the RBCs is rapidly digested and the iron may reutilize. Important pathogenic component in the development of clinical disorders in the chronic hemorrhage appears anemia and hemic type of hypoxia.

In pathophysiological and clinical manifestations dominate following signs:

- insufficiency of tissue respiration due to the development of hemic and tissue hypoxia;
- disorder of tissue metabolism;
- acid-base disbalance (non-respiratory acidosis);

- iron-deficiency anemia;
- disorder of the bone marrow due to its chronic suffering from hypoxia;
- development of chronic posthemorrhagic anemia.

CHAPTER 2
HEMOPOIESIS

There are three periods of blood formation: mesoblastic, hepatic, medullar.

Mesoblastic phase

2-3 weeks after fertilization 3 layer development (ecto, meso, and endoderm). Mesoderm gives rise to «blood islands» with basophilic cells. Central basophilic cells detach as peripheral cells of form vessels allowing primitive erythroblasts — megaloblasts (megaloblastic type of hematopoiesis) to circulate between the yolk sack and embryo. Formation of blood islands occurs in multiple locations. By the end of this period, the first elements of normoblastic hematopoiesis and WBC appear extravascularly.Yolk sac production disappears by 10th week.

Hepatic phase

Liver develops starting at 5weeks. Blood cells begin developing in liver from migration of yolk sac blood island cells and establishment of new colonies. Hematopoiesis by megalo-, normo-, myelo-, lympho-, monoblast and megakaryoblast types derived from the liver extravascularly. Spleen begins developed in 10th week providing new sites for blood cell development. Hematopoietic activity starts to pick up in lymph nodes, thymus, and bone marrow. By the beginning of the 4th month gradually disappears megaloblastic type of hematopoiesis.

Medullary (Myeloid) phase

Starts in the 4th month and becomes dominant by 7 months. By birth, the medullary cavities of the bones are the major site of hematopoiesis, with virtually every bone contributing in this proliferative process and providing mature functional hematopoietic cells to the peripheral circulation. Hepatic hematopoiesis dwindles, persisting only in widely scattered foci that become inactive soon after birth. Lymphoid hematopoiesis continues in the thymus, lymph node.

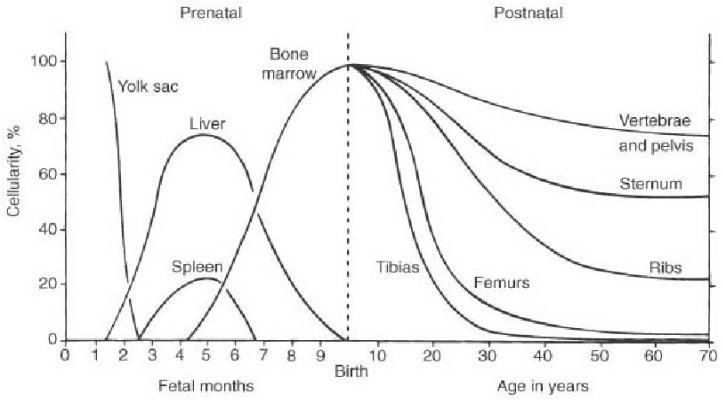


Figure 1 – Sites of hematopoiesis during fetal development and after birth (R. Hoffman et al., 2009)

Hematopoiesis is performed extravascularly in the bone marrow, lymphatic glands, thymus, spleen, gut-associated lymphoid tissue, mucosa-associated lymphatic tissue. Erythrocytes are formed on a normoblast type, granulocytes (neutrophiles, eosinophiles and basophiles) — on a myeloblas type, lymphocytes — on a lymphoblast type, monocytes — on a monoblast type, thrombocytes — on a megakaryoblast type of hematopoiesis.

All blood cells are classified into three large sections: hematopoietic progenitor (stem) (2.1 % by total weight of blood cells), intermediate (25–40 %) and mature (60–75 %). Within these three sections all blood cells are further divided into six classes (table 2).

Table 2 — Sections and classes of hemopietic cells

Sections	Classes
Hematopoietic progenitor	I — pluripotent hematopoietic stem cells
	II — plurioligopotent committed progenitor cells
	III — monooligopotent committed progenitor cells
Intermediate	IV — blasts
	V — maturing cells
Mature	VI — mature cells

I. Pluripotent hematopoietic stem cells rounded mononuclear, size 8–10 mcm, similar in morphology of the bone marrow lymphocytes with high nuclear-cytoplasmic ratio, which does not have the ability to adherent and phagocytic activity. From one stem cells (which started the differentiation) may be formed about 1 million RBC and 100 thousand granulocytes and macrophages.

II. Plurioligopotent committed progenitor cells (semi-stem). This class mainly includes myelopoietic progenitor cells — CFU-GEMM (colony-forming unit granulocyte-erythrocyte-monocyte (macrophage)-megakaryocyte). Also, this class includes progenitor cells that are more limited in differentiation, ie CFU-GM — capable to forming mixed colonies of two kinds of cells, such as granulocytes and macrophages.

III. Monooligopotent committed progenitor cells

Give rise to single cells of myeloid lineage. These include CFU-G — granulocyte progenitor cells and more mature: CFU-N (neutrophilic), CFU-Eo (eosinophilic) and CFU-Ba (basophilic including mast cells); CFU-M (monocytic), CFU-Mk (megakaryocytic). RBC progenitor cells are BFU-E (erythroid bursts forming units) immature (insensitive to erythropoietin) and mature BFU-E (sensitive to erythropoietin). This class also includes Pre-T and Pre-B cells.

IV. Blasts

Blasts are actively proliferating cells, recognized by immunophenotypic and morphological and cytochemical features, that allow to differentiate them by using the method of differential staining. These include myeloblasts, monoblasty, megakaryoblasts, erythroblasts, lymphoblasts.

V. Maturing cells

Maturing cells are not yet fully differentiated, but some of them are already losing the ability to proliferate. Proliferating cells of this class: granulocytic lineage — promyelocytes, neutrophilic, eosinophilic and basophilic myelocytes; promonocytes; promegakaryocytes; megakaryocytes; erythroid cells — pronormoblasts, basophilic and polychromic normoblasts; prolymphocytes. Non-proliferating cells are neutrophilic, eosinophilic and basophilic metamyelocytes and band granulocytes, oxiphylic normoblast and reticulocyte.

VI. Mature cells

Mature cells are nonproliferative specialized blood cells that perform well-defined functions in the body. They are represented by a segment-nuclear neutrophils, eosinophils and basophils, mast cells, monocytes, platelets, RBC, T and B lymphocytes, NK cells.

In tissues mature monocytes are transformed into macrophages. B cells are capable of differentiating consistently into plasmablasts, proplasmacytes and plasma cells.

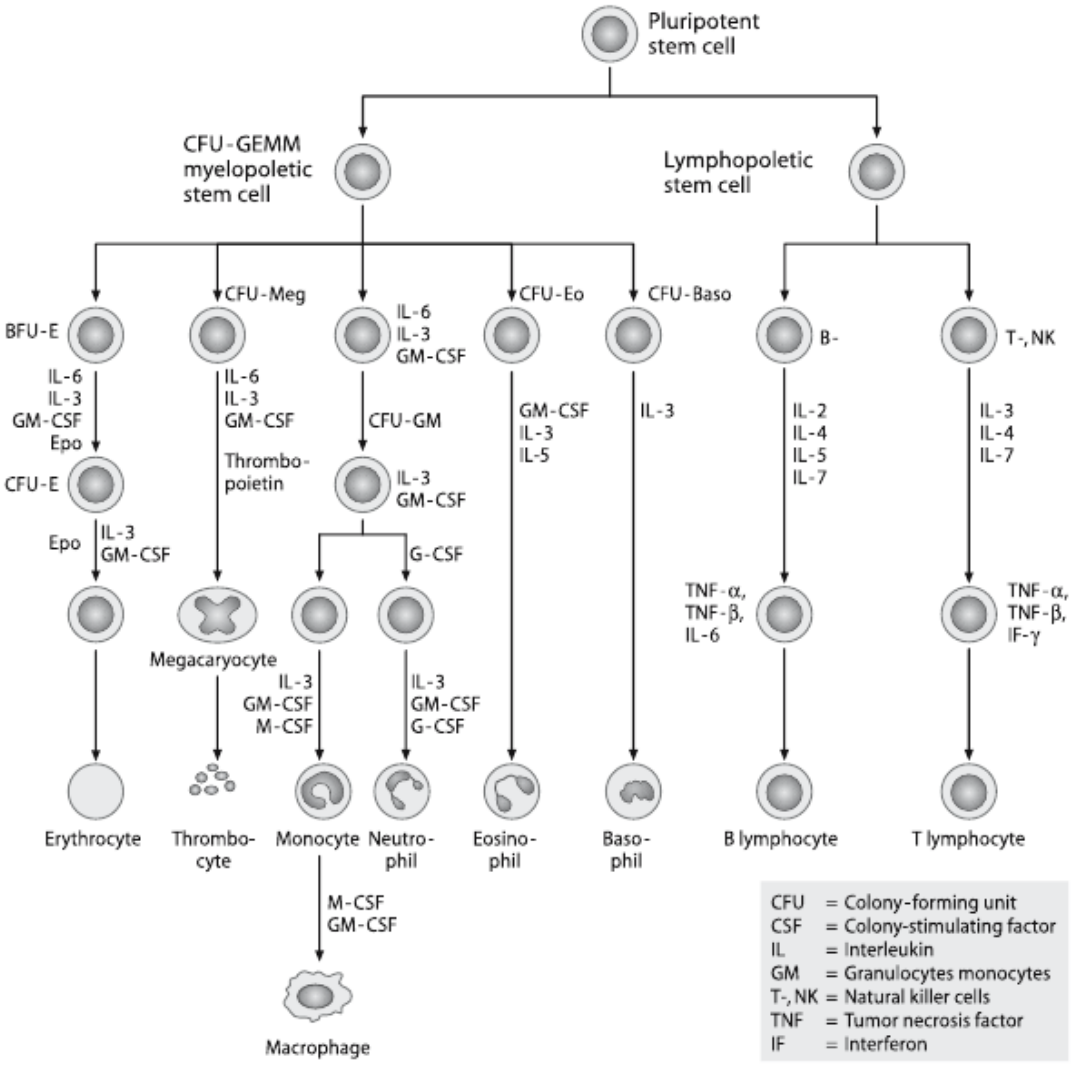


Figure 2 — Scheme of medullary hemopoiesis (H. Lëoffler et al., 2005)

CHAPTER 3
DISORDERS IN ERYTHROCYTE SYSTEM

Erythrocytes (RBC): occupy about 40–45 % of the total blood volume or 30 ml/kg body weight.

ERYTHROPOIESIS

There are megalo- and erythroblast types of hemopoiesis. First morphologically recognizable cell of erythron is erythroblast.

Further stages of erythroid maturation in **erythroblastic erythropoiesis** are following: erythrocytoblast → pronormocyte → basophilic normocyte → polychromatophilic normocyte → orthochromatophilic normocyte → reticulocyte → erythrocyte.

The cell becomes smaller, nucleus is shrinked and in ortochromatophilic erythroblasts, it is expelled from the cell. A change in cytoplasm stainability (basophilia-acidophilia) as polysomes are reduced, hemoglobin is synthetised. Reticulocyte still contains some organeles (polysomes and mitochondria). From single erythroblasts as a result of mitosis appear 16 to 32 reticulocytes. The cycle time of erythroblasts to reticulocyte ranges from 3–4 to 5–7 days. At first reticulocyte matures within the BM (approximately 2–3 days) and then the more mature reticulocytes pass in peripheral blood. Mature red blood cells normally in adults have a lifespan 100–120 days, in full-term newborns — 60–70 days and preterm — 35–50 days.

Megaloblastic erythropoiesis: promegaloblast → basophilic megaloblast → polychromatophilic megaloblast → orthochromatophilic megaloblast → megalocyte.

RBCs production is stimulated by erythropoietin on erythroid progenitors in the bone marrow. Erythropoietin is a secreted glycoprotein produced by renal peritubular cells in response to hypoxia.

Hemoglobin — an oxygen-binding molecule produced only by erythroid cells, is composed of four globin chains (two alpha and two non-alpha, either beta or gamma) each of which binds one heme molecule. The three hemoglobin variants normally seen in healthy adults are hemoglobins A ($\alpha 2\beta 2$), A2 ($\alpha 2\delta 2$), and F ($\alpha 2\gamma 2$).

Heme, the oxygen-carrying prosthetic group of hemoglobin, is a Fe^{2+}-containing tetrapyrolle that functions in electron exchange. Heme synthesis is taken place in both cytoplasm and mitochondria. The 1rst step is conversion of glycine and succinyl coenzyme A to delta aminolevulinic acid by the enzyme aminolevulinic acid (ALA) synthase, with pyridoxine (vitamin B6) as a cofactor. The last (and rate-limiting) step is the addition of Fe^{2+} to protoporphyrin IX by the mitochondrial enzyme ferrochelatase to form heme.

CHANGES OF ERYTHROCYTES

Changes of erythrocytes are:

- anisocytosis — change in the size of RBCs;
- poikilocytosis — change in the form of RBCs (see table 3);
- presence of pathological inclusions (see table 4);
- change in the staining of RBCs (contents of Hb).

Anisocytosis

Measuring the size of RBC can be obtained by Price-Jones curve of RBC size distribution in the peripheral blood (figure3).

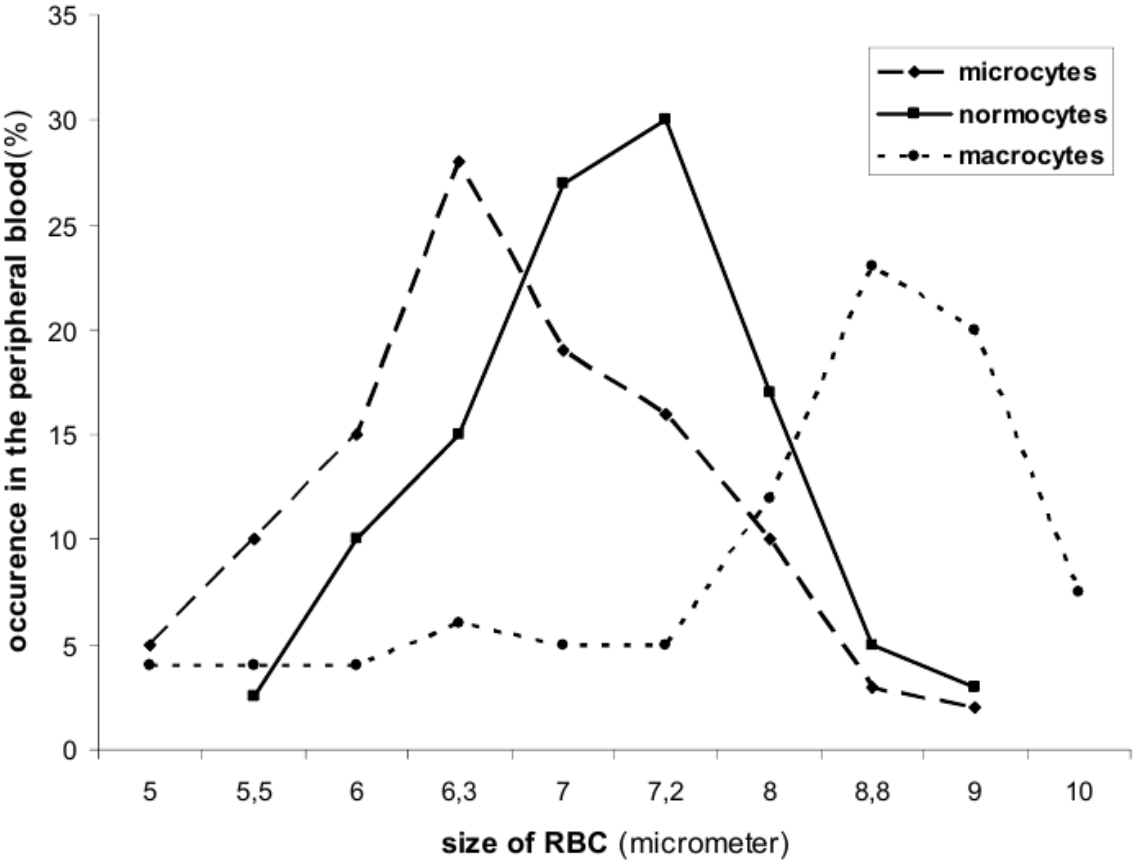


Figure 3 — Price-Jones curve

Variability in size of RBC may be observed also in healthy humans (physiological anisocytosis). In healthy people, up to 5–7 % of all RBC may have smaller or bigger diameter than the mean value.

Normocytes — are RBCs with reference values of size, shape and content of hemoglobin and diameter of RBC 7,2–8,3 mcm.

Microcyte — diameter of RBC < 7,2 mcm.

Macrocyte — diameter of RBC from 8.3 to 12 mcm.

Megalocyte — diameter of RBC 12–15 mcm.

Table 3 — Pathological forms of RBCs

RBC Type	Description	Underlying Change	Disease States
Acanthocyte (spur cell)	Irregularly speculated cells with projections of varying length and dense center	Altered cell membrane lipids	Abetalipoproteinemia, cirrhosis, hepatic necrosis, pyruvate kinase deficiency, uremia, infantile pyknocytosis
Bite cell (degmacyte)	Smooth semicircle taken from one edge	Heinz body «pitting» by spleen	G6PD deficiency, druginduced oxidant hemolysis
Echinocyte (burr cell or crenated cell)	Cells with short, evenly spaced spicules and preserved central pallor	May be associated with altered membrane lipids	Usually artifactual; seen in uremia, bleeding ulcers, gastric carcinoma, artifact
Ovalocyte (elliptocyte)	Ellipticall-shaped cell	Abnormal cytoskeletal proteins	Hereditary elliptocytosis, megaloblastic anemia, myelofibrosis, refractory normoblastic anemia, thalassemia

RBC Type	Description	Underlying Change	Disease States
Helmet cells	These are sharp-angled, helmet-looking distorted, fragmented cells	Mechanical destruction micro-vasculature by fibrin strands mechanical damage or prosthetic heart valve	DIC, microangiopathic hemolytic anemia, thrombotic thrombocytopenic purpura (TTP), prosthetic heart valves, severe valvular stenosis, malignant hypertension
Knizocytes (triconcave Er)	RBCs have a «handle»	Mechanical destruction	Occur mainly in hemolytic anemias
Schistocyte	Distorted, fragmented cell, two or three pointed ends	Mechanical destruction micro-vasculature by fibrin strands mechanical damage or prosthetic heart valve	Microangiopathic hemolytic anemia, DIC, TTP, prosthetic heart valves, severe burns, HUS, giant hemangioma, metastatic carcinoma, malignant hypertension, eclampsia (toxemia of pregnancy), vasculitis, macroangiopathic hemolytic anemia
Sickle Cell (drepanocyte)	Bipolar, speculated forms, sickle-shaped, pointed at both ends	Molecular aggregation of hemoglobin S	Sickle cell disorders excludes S trait
Spherocyte	Spherical cell with dense hemoglobin and absent central pallor; usually decreased in diameter	Decreased membrane redundancy	Hereditary spherocytosis, artifact, autoimmune hemolytic anemia, acute alcoholism, hemoglobin c disease, following severe burn injury, hemolytic transfusion reactions, severe hypophosphatemia, acute oxidant injury: hexose monophosphate shunt defect
Stomatocyte	Mouth or cuplike deformity	Membrane defect with abnormal cation permeability	Hereditary stomatocytosis, immunohemolytic anemia
Target cell (codocyte)	Target-like appearance hypochromic with central hemoglobin	Increased redundancy of cell membrane	Liver disease, postsplenectomy, thalassemia, hemoglobin C disease, iron deficiency
Teardrop cell (dacrocyte)	Distorted, drop-shaped cell	Mechanical distortion of red cell	Myelofibrosis, myelophthisic anemia extramedullary hemopoiesis, severe hemolytic anemia, erythroleukemia
Xerocytes	Shrink flat RBC	Defects in membrane permeability, dehydrate, becoming rigid	Tumors, inherited disorders

Table 4 — Pathological inclusions in erythrocytes

RBC inclusions	Description	Underlying Change	Disease States
Basophilic stippling	Punctate basophilic inclusions, dispersed blue granulations	Precipitated ribosomes, mitochondria	Lead intoxication, thalassemia, arsenic poisoning, thalassemia, sideroblastic anemia, hemolytic anemia, severe anemia, unstable hemoglobin, pyrimidine 5'-nucleotidase deficiency
Pappenheimer bodies (siderocytes)	Small, dense basophilic granules	Iron-containing granules, mitochondrial remnant or siderosome, iron granules, may encircle the nucleus	Sideroblastic anemia, sideroachrestic anemias, postsplenectomy severe hemolytic anemias, lead poisoning, and pernicious anemia
Howell-Jolly	Small (1 mm) dense,	Nuclear fragment	Postsplenectomy, hemolytic ane-

bodies	perfectly round basophilic	containing aberrant chromosomes	mia, megaloblastic anemia
Cabot rings	Ring or figure-eight strand stained purple	Spindle remnant	Lead toxicity, pernicious anemia, hemolytic anemia
Heinz bodies	Round blue precipitates of hemoglobin in RBC detected by supravital staining	Aggregates of denatured hemoglobin	Postsplenectomy, megaloblastic anemia, oxidative hemolytic anemia (G6PD Deficiency)

Change in the staining of RBC (contents of Hb):

Hyperchromic — the RBCs are intensively colored.

Hypochromic — with pale staining. The cells, which have normal diameters, are conspicuous for their paucity of hemoglobin, which may form only a thin peripheral rim (anulocytes).

Polychromatophilia is an ability to perceive the acidic and basic dyes, RBCs painted in color from blue to grayish pink. They arise as a result of insufficient accumulation of hemoglobin in RBCs with the remnants of basophilic substance.

RBCs indices

1. Mean corpuscular volume (MCV)

MCV measures only average cell volume. The value is expressed in volume units (femtoliters, fl). The normal range is 80–97 fl. The formula for the calculation is:

$$MCV = \frac{Ht}{RBCs}$$

Where: Ht — hematocrit rate in %, RBC — the number of red blood cells in millions of 1 mm^3 of blood.

Normocytic refers to blood with a normal MCV. The RBCs are microcytic when the MCV is low than 80 fl. Microcytosis is to look for iron deficiency or thalassemia, anemia of chronic disease, vitamin C and copper deficiencies.

RBCs are macrocytic when the MCV is high. MCV 100–110 fl has a clinical association with alcohol, liver disease (with and without Alcoholism), drug therapy (HIV, oncology and epilepsy), reticulocytosis due to hemolytic anemia; MCV > 110 fl — megaloblastic anemia due to B_{12} or folate deficiency, myelodysplastic syndrome.

2. Mean corpuscular hemoglobin (MCH)

The MCH represents the mean mass of hemoglobin in the RBC and is expressed in the mass unit, pictograms (pg). The formula for the calculation is:

$$MCH = \frac{Hb}{RBC}$$

Where: Hb — count of hemoglobin in blood (g/l), RBC — the number of erythrocytes in 1 liter of blood.

The normal range is 27–31 pg. Elevated MCH is associated with macrocytic anemia. Diminished MCH is associated with microcytic anemia. Hyperlipidemia may give a false elevation of the MCH

3. Mean corpuscular hemoglobin concentration (MCHC)

This is the mean concentration of hemoglobin in the red cell. The formula is:

$$MCHC = \frac{Hb}{Ht}$$

Where: Ht — hematocrit rate in %, Hb — count of hemoglobin in blood (g/l).

Cells with normal, high, and low MCHC are referred to as normochromic, hyperchromic, and hypochromic, respectively. These terms will be important in anemia classification.

4. Red cell distribution width (RDW)

Indicator of heterogeneity of RBCs by volume is characterizing the degree of anisocytosis. At the same time RDW index characterizes fluctuations of cell volume within populations and is not related to the absolute value of the RBCs volume. Therefore, the presence of RBCs in the blood with a modified but quite uniform size (eg microcytes), RDW values may be within normal limits. Units: % — the percentage erythrocyte volume deviations from the average value in the population (% variation). In norm — 11,5–14,5 %.

Red blood cells normally distributed in diameter in a so-called Price-Jones curve (see figure 3).

PHYSICO-CHEMICAL PROPERTIES OF BLOOD

Blood viscosity

The viscosity of blood is determined in relation to the viscosity of water, and depends on blood cell (mainly RBC) and plasma proteins. If we take the viscosity of water per 1, the average relative viscosity of blood in a healthy adult is 4,5 (3,5–5,4) and plasma viscosity — 2,2 (1,9–2,6). In this case, the viscosity of the venous blood is higher than the arterial, which is associated with the arrival of the red blood cells of carbon dioxide causes an increase in cell size.

Reasons for the increase in blood viscosity:

- age (blood viscosity increases with age);
- excessive protein diet;
- dehydration;
- polycythemia;
- emptying of depot (spleen, liver, lungs, bone marrow, etc.);
- violation of deformability and aggregation of erythrocytes;

• activation of coagulation factors.

Osmotic blood pressure

The osmotic blood pressure — is the force with which the solvent (for blood is water) passes through a semipermeable membrane from a less concentrated to a more concentrated solution. The osmotic pressure of the blood is important in the regulation of the water distribution between tissues and blood vessels, cells and interstitial fluid. The functions of the body's cells can be carried out only if it’s relative stability, which is provided by neurohumoral mechanisms – antidiuretic and antinatriuretic systems.

The osmotic pressure of blood may affect the products of digestion of proteins, fats and carbohydrates are absorbed into the blood and lymph systems, as well as low molecular weight products of cell metabolism.

Erythrocytes osmotic resistance

Resistance (resistance) of red blood cells — the ability to withstand a variety of their destructive effects: osmotic, mechanical, chemical, physical, and etc.

1. *Isotonic Solutions*

Isotonic solution is the solutions having the same effective osmolality (tonicity) as body fluids (0,9 % sodium chloride solution, 5 % glucose solution). The osmotic is in equilibrium between inside and outside the cell across the cell membrane.

2. Hypertonic Solutions

Hypertonic solutions — is the solutions having greater effective osmolality than the body fluids like 2 % sodium chloride solution. When RBCs are placed in hypertonic solution, water moves out of the cells (exosmosis) resulting in shrinkage of the cells (crenation).

3. Hypotonic Solutions

Hypotonic solutions — is the solutions having less effective osmolality than the body fluids. For example is 0.3 % sodium chloride solution. When the RBCs are taken in hypotonic solution, water moves into the cells (endosmosis) resulting in swelling and rupture (hemolysis) of the cells.

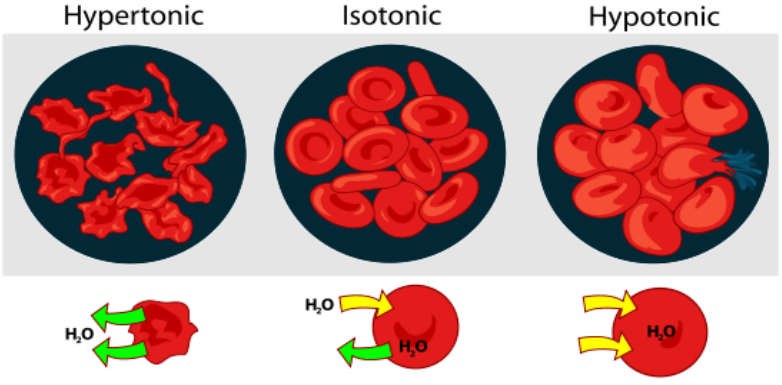


Figure 4 — Effect of hypertonic and hypotonic solutions on red blood cells

Decrease in osmotic resistance of erythrocytes (increase the minimum and maximum indicators of resistance) is observed at:

- autoimmune hemolytic anemia caused by thermal antibodies;
- hemolytic disease of the newborn;
- hereditary microspherocytosis and stomatocytosis;
- B_{12} deficiency anemia;
- toxicosis;
- bronchopneumonia, violations of the functions liver and spleen;
- hemoblastosis.

Increase in osmotic resistance of erythrocytes observed in:

- obstructive jaundice;
- polycythemia;
- iron deficiency anemia;
- thalassemia;
- sickle cell anemia;
- after massive blood loss.

Total protein

In human plasma contains about 100 different proteins. By electrophoretic mobility can be roughly divided into five fractions: albumin, α1-, α2-, β- and γ-globulins. Separation into albumin and globulin is initially based on the difference in solubility: albumins are soluble in pure water, and globulins — only in the presence of salts. Main mass of the plasma protein synthesized in the liver. Liver cells (hepatocytes) are involved in the synthesis of albumin, fibrinogen, α- and β-globulins and components of coagulation system. Most of the β- and γ-globulins synthesized in cells of the immune system (lymphocytes).

Plasma proteins play important physiological roles in the body:

- maintain viscosity, the fluidity of blood;
- determine the volume of blood in the bloodstream;
- keep the blood cells in suspension;
- carry multiple transport of exogenous and endogenous substances (hormones, minerals, lipids, pigments, and other. Biologically important compounds);
- regulate the constancy of blood pH;
- are coagulation factors;
- involved in immune responses (immunoglobulins, opsonins, acute phase proteins).

Changes in the concentration of total protein may be physiological, relative and absolute.

Physiological hypoproteinemia may occur in young children, women during pregnancy (especially in the third trimester), lactation, prolonged bedrest.

The relative changes in protein content observed in the increase (decrease) in circulating blood volume. Hydremia (load water, «water» poisoning) results in a relative hypoproteinemia, and dehydration (dehydration) — relative to hyperproteinemia.

Absolute hypoproteinemia is observed at:

- insufficient intake of proteins in the body as a result of starvation, malnutrition, narrowing (stricture) of the esophagus, disorders of integrity and function of gastrointestinal tract and other conditions involving deterioration of digestion and absorption of proteins;
- violation of protein synthesis in the body due to violation of the protein synthetic function of the liver (cirrhosis, hepatitis, tumor metastasis to the liver etc.);
- increased body protein loss due to acute and chronic bleeding, severe burns, chronic renal disease with nephrotic syndrome;
- strengthening catabolism (decomposition) of the protein due to prolonged hyperthermia, thermal burns, hyperthyroidism, prolonged physical activity, cancer;
- redistribution of protein (protein output from the vascular bed and the formation of exudates and transudate).

Absolute hyperproteinemia (relatively rare) is observed at:

- acute and chronic infectious diseases (due to globulin);
- autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, rheumatism, etc.);
- oncological diseases with pathological overproduction of proteins — paraproteinemia (multiple myeloma (plasmacytoma), Waldenstrom's macroglobulinemia).

Erythrocyte Sedimentation Rate (ESR)

Erythrocyte sedimentation rate (ESR) — separation rate stabilized with anticoagulants blood into two layers: upper — transparent plasma and lower — sedimented erythrocytes. The main influence on the ESR exercises aggregation of RBCs, force of which depends on surface charge of them and plasma concentrations of asymmetric molecules (proteins). Aggregation leads to the formation of agglomeration and adhesion of erythrocytes («coin columns»), moves to the lower layers on standing blood.

The ESR depending on:

1. Changing the ratio of different fractions of blood proteins: decrease in concentration of albumin or/and increase in levels of coarse protein (α-globulins, γ-globulins, fibrinogen) elevated ESR.
2. Increase in volume, number and diameter of RBC slows down the ESR, their decrease in elevate ESR.
3. Cholesterol adsorbed on red blood cells accelerates ESR; lecithin, bile acids and pigments, slow down the ESR.
4. The pH of the blood: alkalosis elevates ESR, acidosis — slows down the ESR.

5. The viscosity of the blood. Dilution anemia leads to elevating ESR, an increase in blood viscosity (dehydration) — ESR slow down.

Table 5 — Main reasons of ESR changes

Disease	ESR	Reasons
Acute inflammation	↑	Fibrinogen↑, albumen↓
Liver cirrhosis	↑	Ig↑, albumen↓↓
Nephrotic syndrome	↑	albumen↓↓, RBC↓
Leucemia	↑	Fibrinogen↑, albumen↓, RBC↓
Monoclonal gammapathy	↑	Ig↑↑
Anemias	↑	RBC↓
Primary and secondary polyerythremias	↓	RBC↑
Cryoglobulinemia	↓	Monoclonal Ig↑

Great influence on the ESR provides certain medications and therapeutic interventions. Thus, the acceleration of erythrocyte sedimentation is observed in specific and nonspecific irritant therapy, vaccine therapy, blood transfusions, long reception of soda, vitamin A, contraceptives, etc. The slowdown of ESR is observed in the reception of salicylic, mercury and calcium preparations, diuretics, hypnotics and anti-malarial drugs.

ANEMIA

Anemia is a clinical-hematological syndrome that characterized by decrease in hemoglobin or/ and erythrocytes in the blood unit.

Table 6 — Classification of anemia

According to cause:	primary secondary
According to the rate of development:	acute chronic
According to mechanism:	post-hemorrhagic hemolytic dyserythropoietic
According to hemopoietic type:	erythroblastic megaloblastic
According to regeneratoric ability of bone marrow:	regeneratoric — 0,2–1 % of reticulocytes hyperregeneratoric — > 1 % of reticulocytes hyporegeneratoric < 0,2 % of reticulocytes aregeneratoric (aplastic) — 0 % of reticulocytes
According to color index:	normochromic — 0,85–1,05 hyperchromic — > 1,05 hypochromic — < 0,85
According to Er size:	normocytic — 7,2–8,3 mcm microcytic — < 7,2 mcm macrocytic — > 8,3–12 mcm

	megalocytic — 12–15 mcm
According to severity:	mild — Hb > 100g/l; Er > $3{,}0 \cdot 10^{12}$/l medium — Hb: 100–66g/l; Er: $3{,}0–2{,}0 \cdot 10^{12}$/l severe — Hb < 66g/l; Er < $2{,}0 \cdot 10^{12}$/l

DYSERYTHROPOIETIC ANEMIAS

Dyserythropoietic anemias:

- **Disorders of erythropoiesis:**

✓ Deficiency anemias (B_{12}-, Fe-, folic acid).

✓ Achrestic anemias (due to inability to absorb by bone marrow hematopoietic agents (B_{12} achrestic, sideroachrestic).

- **Aplastic anemia** (due to damage of bone marrow by diferent factors (ionizing radiation, toxic drugs)).
- **Metaplastic anemia** (during leukemia, metastasis of tumors in bone marrow).

Iron deficiency anemia

Iron deciency is the most common cause of anemia world-wide. Iron balance is regulated by several conditions including the following:

- amount of iron ingested;
- amount of iron absorbed;
- RBCs formation using recycled and new iron;
- iron stores;
- iron loss through blood loss or other sources.

Total body iron store is about 4 g. Normal diet provides approximately 15 mg of iron in day, of which 5–10 % is absorbed in duodenum and upper jejunum. About 15–30 % of total iron is stored for later use, mainly in the reticuloendothelial system and liver parenchymal cells in the form of ferritin. Iron is important for Hb formation and also myoglobin, cytochromes, cytochrome oxidase, peroxidase, catalase.

Heme, the oxygen-carrying prosthetic group of hemoglobin, is a Fe^{2+}-containing tetrapyrolle that functions in electron exchange. Heme synthesis is taken place in both cytoplasm and mitochondria. The first step is conversion of glycine and succinyl coenzyme A to delta aminolevulinic acid by the enzyme aminolevulinic acid (ALA) synthase, with pyridoxine (vitamin B_6) as a cofactor. The last (and rate-limiting) step is the addition of Fe^{2+} to protoporphyrin IX by the mitochondrial enzyme ferrochelatase to form heme.

Etiology of the iron deficiency anemia

The iron deficiency anemia may occur as a result of an iron-deficient diet, inadequate intestinal iron absorption, chronic blood loss (menorrhagia or gastrointestinal) or intravascular hemolysis with hemoglobin loss in urine (hemoglobinuria).

Table 7 — Causes and mechanisms of development of iron deficiency

Groups of etiological factors	Characteristics	Mechanisms
Special periods of life	Premature and newborn children Children first years of life	Lack of initial level of iron
	Intensive growth (puberty) pregnancy lactation	Increased iron consumption
Pathological conditions	Chronic blood loss: frequent therapeutic phlebotomy, blood donation; Cardiovascular disease (hypertension, hemorrhagic telangiectasia, etc.); Gastrointestinal pathology (esophageal varices, diaphragmatic hernia, gastric and duodenal ulcers, ulcerative colitis, diverticulosis, etc.); Urogenital system (alcohol nephropathy, renal tuberculosis, nephrolithiasis, polyps and cancer of bladder, profuse menorrhagia, endometriosis, uterine fibroids, etc.); Respiratory system (lung cancer, tuberculosis, bronchiectasis, etc.); Blood diseases (leukemia, aplastic anemia, etc.); Pathology of the hemostatic system (autoimmune thrombocytopenia, hemophilia, DIC, etc.)	Increased iron lose
Pathological conditions and diseases	Pathology of the gastrointestinal tract: resection of the stomach and intestines, gastric hyposecretion, chronic enteritis, dysbacterioses, helminthic invasion, etc.	Impaired iron absorption
	Hereditary atransferrinemia Acquired hypotransferrinemia (violation of the liver protein synthesis)	Impaired iron transport
	Alcoholism	Combination of factors: insufficient intake of iron, impaired iron absorption and transport, iron loss
Impaired iron absorption	Irrational nutrition: starvation, vegetarian diet, artificial feeding of infants	Insufficient intake of iron
	Excessive exercise	Increased consumption of iron

Pathogenesis

Hemoglobin — an oxygen-binding molecule produced only by erythroid cells, is composed of four globin chains (two alpha and two non-alpha, either beta or gamma) each of which binds one heme molecule. Four iron molecules are needed in each hemoglobin unit. The main role for iron is as the ion in the

center of the body's oxygen-carrying molecule, heme. Iron, held stably in the ferrous form by the other atoms in heme, reversibly binds oxygen.

When there is iron deficiency, the final step in heme synthesis is interrupted. In this step, ferrous iron is inserted into protoporphyrin IX by the enzyme ferrochelatase. When heme synthesis is interrupted, there is inadequate heme production. A result of heme deficiency is elevation of heme-regulated translational inhibitor activity, which inhibits a key transcription initiation factor for heme synthesis. Thus, there are less heme and fewer globin chains available in each red cell precursor. This decrease in the hemoglobin concentration of the blood is directly causes anemia.

Clinical manifestations

There are 2 main syndromes during iron deficiency anemia: general symptoms of anemia and cideropenic syndrome.

General symptoms of anemia:

• weakness;
• dizziness;
• impaired memory and attention;
• pale skin with a greenish tinge.

Sideropenic syndrome:

• trophic disorders of the skin, mucous;
• cheilitis (inflammation around the lips);
• atrophic glossitis;
• koilonychias (spooning of the nail beds) ,angular stomatitis;
• pica (abnormal craving for unusual substances such as dirt, ice, or clay);
• sideropenic dysphagia (Plummer-Vinson syndrome);
• iron deficiency in infants may result in developmental delays and behavioral disturbances.

Stages of iron deficiency anemia

Stage 1: iron stores depleted; test for absence of stainable bone marrow iron, decreased serum ferritin level, increased TIBC;

Stage 2: iron-deficient erythropoiesis; test for slight microcytosis, slight decreased hemoglobin, decreased transferrin saturation;

Stage 3: iron deficiency anemia; test for decreased serum iron, decreased serum ferritin, increased TIBC, decreased transferrin saturation.

Laboratory tests

Peripheral blood smear:

• Hb and Ht are decreased usually to moderate levels, the MCV and MCHC are decreased;

• hypochromia, poikilocytosis; microcytosis and normocytic cells with decreased central area of pallor;

• decrease in color index below 0.8;

• reticulocytes in normal or slightly increased, with the progression of IDA their number decreases;

• often developing neutropenia (by reducing the content of iron-containing enzymes in leukocytes);

• ESR is normal or slightly increased;

• platelet count may be slightly elevated (on the background of bleeding).

Biochemical analysis of blood:

• serum iron in severe IDA drops to 5,4–1,8 mmol/l at norm 12,5–30,4 mmol/l men, women 10–15 % lower;

• increased concentration of transferrin;

• decrease of transferin saturation with iron less then 20 % (in norm 30–50 %), it means a decrease of iron transport to the bone marrow;

• decrease of serum ferritin less then 12 mkg/l (in norm 12–200 mkg/l);

• increased concentration of TIBC;

• decreased synthesis of some iron-contacting enzymes in erythrocytes (catalase, glutathionperoxidase) results in erythrocytes increased sensitivity to hemolyzing effect of oxidizers (the lifetime of RBCs gets reduced to 20–30 days);

• increase in the content of soluble receptors to transferritin in serum;

• increase in free protoporphyrin IX in erythrocytes.

Bone marrow examination:

• normoblastic hyperplasia with impaired hemoglobinisation (predominance of basophilic and polychromic normoblasts while reducing oxyphylic);

• decrease in the index of normoblast maturation;

• decreased a count of sideroblasts until their complete absence;

• myeloid and megakaryocyte germs are not changed.

Anemia of chronic disease

Anemia of chronic disease is a mild to moderate anemia associated with chronic inflammation or infection (meningitis, pneumonia, tuberculosis, osteomyelitis, syphilis, fungal infections etc.), systemic connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, etc.) and tumors (multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's disease, lung cancer, breast cancer etc.). This form of anemia is due to a combination of mildly decreased red cell survival and inadequate erythropoiesis.

***Pathogenesis*:**

1) inflammatory cytokine-mediated activation of splenic and hepatic macrophages and increased binding of antibody and complement to red cells leads to an increased rate of hemolysis;

2) inflammatory cytokines interleukin 1 (IL-1) and tumor necrosis factor alpha (TNFa) inhibit erythropoietin synthesis;

3) inflammatory cytokine IL-6 induces hepatic synthesis of hepcidin (the iron-regulating hormone). Hepcidin binding to the cell membrane iron transporter protein ferroportin leads to ferroportin degradation, blocking the release of iron by macrophages and intestinal cells and preventing the transfer of iron to red cell precursors in the marrow;

4) in patients with malignant tumors, along with the cytokine-mediated inhibition of erythropoiesis, is also associated with metastatic lesions of the bone marrow and myelofibrosis.

The anemia may be hypochromic, microcytic. Serum iron is decreased, TIBC is decreased (blood levels of the negative acute-phase reactant transferrin drop in response to inflammation). Serum ferritin is increased.

Examination of the marrow in anemia of chronic disease reveals numerous iron-laden macrophages and markedly decreased sideroblasts (nucleated red cells with particles of stainable iron).

Anemias associated with internal diseases

Anemias associated with internal diseases include anemias of endocrine, liver and kidney diseases.

Anemias in endocrine diseases: anemia in diseases of the thyroid and parathyroid glands, adrenal glands, gonads, hypopituitarism etc.

The most common pathogenic is depression of erythropoiesis due to deficiency or hypersecretion of several hormones. For example, thyroxine, cortisol, testosterone in very high concentrations cause inhibition of proliferative activity of erythroid precursors.

Anemias in liver diseases occur when the diffuse lesions (cirrhosis, chronic hepatitis, hemochromatosis, etc.). Distinguish the following mechanisms of anemia:

• suppression of hematopoiesis in the bone marrow (due to the direct toxic effects on the hematopoietic progenitor cells of alcohol (in alcoholic liver disease) and endogenous toxins (in violation of neutralizing and clearance liver function); disorders of iron metabolism and deposition of vitamin B_{12} and folic acid in the damaged liver);

• shortening the lifetime of erythrocyte (as a result of direct damaging action of exogenous (alcohol) and endogenous (endotoxemia) toxic products; hypersplenism; disorders of intracellular metabolism of erythrocytes (e.g., $NADP^+$ deficient in the cells) and their ability to deform (due to pathological changes in the cell membrane);

• varical bleeding from the gastrointestinal tract (in liver cirrhosis), nasal, hemorrhoidal and other sites (in insufficient synthesis of coagulation factors due to violations of protein metabolism).

Anemias in kidney disease can be detected in patients with acute glomerulonephritis, interstitial nephritis and chronic renal failure. The pathogenesis of anemia is determined by decreased production of erythropoietin, depression of hematopoiesis in bone marrow (as a result of violations of the proliferative activity of erythroid cells, inhibition of heme synthesis) and shortened lifetime of red blood cell (40–50 days) under the action of toxic products of nitrogen metabolism.

Megaloblastic anemias

The megaloblastic anemias are a group of disorders caused by defects in DNA synthesis. Anemia develops as a result of the production of enlarged erythroid precursors that are destroyed within the marrow (ineffective erythropoiesis) as a result of their inability to undergo normal DNA replication and cell division. Folate and vitamin B 12 are both essential for DNA replication and are therefore required for normal hematopoiesis.

Vitamin B_{12} deficiency anemia

B_{12} (cobalamin) is a cofactor involved in DNA synthesis.

Etiology of vitamin B_{12} deficiency anemia

In the diet, cobalamin is found only in animal products. Dietary deficiency, which occurs only in strict vegetarians, is rarely.

Pernicious anemia (Addison-Birmer's disease) is the most common cause of vitamin B_{12} deficiency. The fundamental defect in pernicious anemia is severe gastric atrophy, with loss of all gastric secretions including intrinsic factor, the presence of which is necessary for absorption of vitamin B_{12}. About 90 % of patients with pernicious anemia have antiparietal cell IgG antibodies in the serum, while 60 % have serum anti-intrinsic factor antibodies. These antibodies are not specific for pernicious anemia.

Table 8 — Causes of Vitamin B_{12} deficiency anemia

Causes	Mechanisms	Clinical conditions
Inadequate intake	Dietary deficiency (rare)	Strict vegetarian, breastfed infants of deficient mothers
Defective absorption	Decreased intrinsic factor	Pernicious anemia (the most common cause), juvenile pernicious anemia, gastrectomy
	Inadequate pancreatic proteases	Pancreatic insufficiency, Zollinger-Ellison syndrome
	Compete with the host for cobalamin	Parasitic or bacterial overgrowth, fish tapeworm
	Mucosal defects	Sprue, surgical resection, amyloidosis
	Drug-induced effect	Colchicine, paraaminosalicylate, neomycin, colestyramine
	Decreased transcobalamin-II	Congenital deficiency
Increased requirements	Increased utilization or loss	Hemolysis, pregnancy, lactation, infancy, intensive growth , hemodialysis
Disorders of metabolism	Inhibition of suppression of enzymes	Heredity enzyme defects, nitrous oxide inhalation (inactivates coenzyme forms of vitamin B_{12})

Pathogenesis

Vitamin B_{12} is freed from binding proteins in food through the action of pepsin in the stomach and binds to salivary proteins called cobalophilins. In the duodenum by the action of pancreatic proteases bound vitamin B_{12} is released. It then bound to intrinsic factor. This complex is transported to the ileum. Ileal enterocytes express intrinsic factor receptors on their surfaces, where it is endocytosed and associated with a major carrier protein (transcobalamin II). Transcobalamin II delivers vitamin B_{12} to the organs (liver, bone marrow, gastrointestinal tract). In addition to this major pathway, there is also a poorly understood alternative uptake mechanism that not or an intact terminal ileum. 1 % of a large oral dose can be absorbed by independent on intrinsic factor way.

Cobalamin accepts a methyl group from methyltetrahydrofolate, which leads to the formation of methylcobalamin and reduced tetrahydrofolate. Cobalamin deficiency depletes stores of reduced tetrahydrofolate and impairs DNA synthesis because of lowered purine production. Tetrahydrofolate is required as the single-carbon donor in purine synthesis.

In DNA synthesis, cobalamin, along with folic acid, is crucial as a cofactor in the synthesis of deoxythymidine from deoxyuridine. Methylcobalamin need for formation of thymidinemonophosphate from uridine monophosphate it lead to disorders in DNA synthesis, production of myelin, neurotransmitters / protein, fatty acid, phospholipid and DNA methylation. Thus deficiency of methylcobalamin lead to development of megaloblastic anemia, disorders of regeneration. Methylcobalamin serves as an essential cofactor in the conversion of homocysteine to methionine by methionine synthase.

Deoxyadenosylcobalamin involved in metabolism of fatty acid and conversion of methylmalonic acid to succinic acid. Accumulation of methylmalonic acid during pernicious anemia facilitates development of dystrophy of dorsolateral columns of the spinal cord, lead to development of funicular myelosis and dysfunction of central nervous system.

Clinical Manifestations

Pernicious anemia is manifested by a triad of symptoms:

1) impairment of hemopoiesis;

2) atrophic changes of the mucus of the gastric intestinal tract;

3) dysfunction of the nervous system.

Anemia is the most commonly encountered abnormality. Typical symptoms are fatigue, dyspnea, dizziness, due to a decreased oxygen-carrying capacity of the blood. High-output congestive heart failure is relatively common, with tachycardia and signs of left ventricular failure.

Gastric intestinal symptoms are less prevalent and include malabsorption diarrhea (more common), and glossitis (most common).

Dysfunction of central nervous system is characterized by appearance of mental disorders (delusions, hallucinations), wobbly gait, paresthesia, pain, numbness

of limbs, paraparesis, occurrence of pathological reflexes, development of funicular myelosis. Funicular myelosis manifests by impaired perception of deep touch, pressure and vibration, disappear of touch sense, decrease or disappear of deep muscle-tendon reflexes, annoying and persistent paresthesias, ataxia of dorsal cord type.

In the serum may be founded low vitamin B_{12}, and elevated levels of homocysteine and methylmalonic acid.

Peripheral blood smear

In peripheral blood is founded hyporegeneratoric, hyperchromic megaloblastic anemia, leukopenia with neutropenia and hypersegmented granulocytes, relative lymphocytosis, thropmbocytopenia. In smear are revealed megaloblasts, megalocytes, macrocytosis, anizocytosis, poikilocytosis, erythrocytes with Jolly's bodies, Kabo's rings and azurophilic granularity, giant hypersegmented neutrophils.

Bone marrow examination

The bone marrow is hypercellular with megaloblastic anemia, dominated by red cell lines (leuko/erythroblastic ratio of 1: 2–1: 3). Type of erythropoiesis with mild to moderate disease severity mixed — normo-megaloblastic (found erythroblasts and normoblasts along with promegalo- and megaloblast). In severe is a megaloblastic erythropoiesis (all the cells of red line are pro-megaloblast and megaloblast), often with signs of degeneration. There is a marked macrocytosis of neutrophils, especially metamyelocytes and polymorphonuclear neutrophils. The number of megakaryocytes is usually not changed, but in severe cases can be reduced.

Biochemical analysis of blood

- decrease of B_{12} in serum and in RBCs;
- increases of methylmalonic acid and homocysteine in serum and urine;
- megaloblastic anemia — usually accompanied by moderate hyperbilirubinemia (due to indirect bilirubin) to 28–47 mmol/l, and an increase in LDH activity (due to the disintegration of intramedullary structures containing a large amount of this enzyme).

Folic acid deficiency

Folic acid deficiency anemia occurs much less frequently than B_{12} deficiency. Folic acid is a water-soluble, heat-labile vitamin. Folate found in meat, liver, vegetable products (spinach, asparagus, lettuce, beans, vegetables, fruits, mushrooms), yeast, milk. More than 50 % of folate is destroyed when the long cooking. To meet the needs of the organism in folate should eat fresh vegetables and fruits. Folate absorption occurs in the duodenum and proximal jejunum.

Table 9 — Etiology of folate deficiency anemia

Causes	Mechanisms	Clinical conditions
Inadequate intake	Dietary deficiency	Alcoholism, starvation, diet without green vegetables, long thermal cooking of food, nurse of infants by gout milk
Increased requirements	Growth, proliferative states, or loss exceeds intake	Pregnancy, lactation, intensive growth of child, increased hematopoiesis (hemolytic anemias, multiply myeloma), malignant diseases, hemodialysis, exfoliative skin disorders, tuberculosis

Causes	Mechanisms	Clinical conditions
Defective absorption	Gastrointestinal abnormalities	Sprue and other small bowel disorders, surgical resection, amyloidosis, congenital malabsorption
	Interference of absorption by drugs	Phenytoin, primidone, phenobarbital, oral contraceptives
Disorders of metabolism	Inhibition of folate metabolism	Inhibitors of dihydrofolate reductase: methotrexate, pentamidine, pyrimethamine; alcohol
	Inherited disorders	Congenital enzyme deficiencies (dihydrofolate reductase), others (rare)
	Disorders of depot in liver	Toxic and viral hepatitis, cirrhosis, hepatocellular cancer

Pathogenesis

Folate is required for synthesis of three of the four bases used for deoxynucleotide synthesis, the two purine bases, adenine and guanine, and the pyrimidine base thymidine. It is the role of folate in thymidine synthesis that is most critical for DNA replication. Methylenetetrahydrofolate is the form required for the conversion of deoxyuridine to thymidine and the production of this form of folate requires vitamin B_{12}. This results in defective DNA synthesis and abnormal growth and maturation of hematopoietic and other rapidly dividing cells.

Deficiency of folate may also cause several complications that affect other organ systems. It is an increased risk of neural and other developmental defects in infants born to folate deficient mothers. Raised homocysteine level has been implicated as an independent risk factor for cardiovascular disease as well as neurodegenerative disease such as Alzheimer — type dementia. As in vitamin B_{12} deficiency, serum homocysteine levels are increased, but methylmalonate concentrations are normal. However, funicular myelosis do not occur.

Picture of peripheral blood and bone marrow corresponds to B_{12} deficiency anemia, changes are less pronounced

Biochemical analysis of blood:

- decrease of folic acid in serum and red blood cells;
- increases of homocysteine in serum and urine; level of methylmalonic acid is normal;
- usually accompanied by moderate hyperbilirubinemia (due to indirect bilirubin) to 28–47 mmol/l, and an increase in LDH activity (due to the disintegration of intramedullary structures containing a large amount of this enzyme).

ACHRESTIC ANEMIAS

Achrestic anemias is developed due to inability to absorb by bone marrow hematopoietic agents (vitamin B_{12}, iron).

B_{12}-achrestic anemia

The development of this anemia is associated with a metabolic disorder of methylcobalamin, resulting in bone marrow hematopoietic loses the ability to

utilize the substance, there is megaloblastic erythropoiesis. Changes in the digestive and nervous systems are not available. Blood picture, as in B_{12} deficiency anemia. The vitamin B_{12} in plasma may be normal or elevated

Sideroahrestical anemia (SAA)

Sideroahrestical (sideroblastic) anemia — is a heterogeneous group of inherited and acquired diseases resulting from violations of porphyrin synthesis or recycling. Deficiency in formation of porphyrins at SAA leads to a violation of iron using.

Classification of sideroblastic anemias:

• Congenital (X-linked, autosomal dominant, autosomal recessive pattern, sporadic (hereditary character is unclear), associated with mitochondrial cytopathy (syndrome Pearson), DIDMOAD syndrome (diabetus insipidus, diabetus mellitus, optic atrophy, deafness; Wolfram syndrome).

• Acquired:

— idiopathic: refractory anemia with ringed sideroblasts (variant of MDS);

— associated with hematologic neoplasia (multiple myeloma, malignant non-Hodgkin's lymphoma, acute leukemia);

— deficiency of vitamin B6;

— medications: azathioprine, isoniazid, melfolan;

— toxic: alcohol, lead, cadmium, nickel poisoning).

The ***pathogenesis*** of SAA is defective enzymes involved in the synthesis of protoporphyrin and heme: 5-aminolevulinate synthase, CPG decarboxylase, CPG-oxygenase, ferrochelatase and others. Activity deficit of listed above enzyme promotes disturbance of protoporphyrin formation at various stages (depending on the level of metabolic block). As a result, the level of protoporphyrin in erythrokaryocytes of BM sharply reduced. Iron cannot connect to the protoporphyrin and incorporated into the structure of hemoglobin. It is deposited in the mitochondria of erythrokaryocytes, forming a large number of ringed sideroblasts. Thus develops ineffective erythropoiesis, intramedullary hemolysis of erythrokaryocytes and as a consequence anemia.

Peripheral blood smear:

• hypochromic microcytic anemia: anisocytosis due to microcytes; poikilocytosis due to target cells;

• reticulocytes in normal or reduced;

• increased siderocytes;

• leukocytes and platelets are normal.

At lead intoxication:

• reticulocytes increased up to 3–8 % (due to RBCs hemolysis);

• basophilic stippling in erythrocytes;

• thrombocytopenia.

Bone marrow examination: hypercellular due to severe hyperplasia of red sprout, increase in erythroblasts, basophilic normoblasts and decreased oxyphylic

normoblast (decrease maturation index of erythrokaryocytes). Increased (up to 70 % in hereditary forms) content sideroblasts (ringed sideroblasts).

Biochemical analysis of blood:

- increase in serum iron (up to 62,7–98,5 mmol/l);
- reduction of TIBC;
- increase in the degree of transferrin saturation (in most patients is almost 100 %);
- increase in serum ferritin.

APLASTIC ANEMIA

Aplastic anemia (AA) is broadly defined as pancytopenia (anemia, low leukocyte and platelet count) with a hypocellular bone marrow.

Classification of aplastic anemias:

1. Congenital (Fanconi's anemia, Shwachman-Diamond syndrome).
2. Acquired:

a. Secondary — resulting from exposure to one of the possible causative exogenous or endogenous factors:

- physical (radiation);
- chemical (drag-induced, industrial toxins — benzene, other aromatic hydrocarbons, pesticides, arsenic);
- biological (hepatitis B virus, parvovirus B_{19}, Epstein-Barr virus);
- pregnancy.

b. Idiopathic.

Depending on the type of bone marrow involvement is distinguish:

- AA with the oppression of all germs of hematopoiesis (Fanconi's anemia, acquired anemias);
- AA with only red germ inhibition (Diamond-Blackfan anemia);
- Neutropenia (congenital dyskeratosis, anemia Shwachman-Diamond-Oski);
- Thrombocytopenia (congenital amegakaryocytic thrombocytopenia).

Congenital Aplastic Anemia

Congenital aplastic anemias are typically associated with dysmorphic physical features, such as growth retardation, limb hypoplasia, and cardiac or renal abnormalities.

Diamond-Blackfan anemia or constitutional pure red cell aplasia, is a results from sporadic abnormalities at chromosome 19ql3. This mutation lead to defects in erythroid precursors that prevent them from responding to growth signals. In 40% anemia is associated with congenital craniofacial, neck, or thumb defects. In all these anemias, the bone marrow shows well-developed granulopoiesis and megakaryopoiesis, but erythropoiesis is (more or less) entirely lacking. The anemia is normochromic and macrocytic.

Fanconi's anemia familial bone marrow failure is characterized by an impaired ability to repair damaged DNA crosslinks. These patients often are associated with other phenotypic abnormalities, such as skin pigmentation, renal or splenic hypoplasia, hypoplastic thumbs or radii, microcephaly, and mental retardation.

Fibroblasts and lymphocytes from these patients have a high incidence of gaps, breaks, chromatid exchanges, and endoreduplication. These patients have an increased incidence of acute myelogenous leukemia.

Secondary Aplastic Anemia

1. *Radiation*

Acute radiation sickness with high-dose whole-body irradiation typically develops marrow aplasia (often fatal) at 3–6 weeks. Chronic exposure to low-dose radiation may result in aplastic anemia, presumably as a result of hematopoietic stem cell injury.

2. Chemical

Drugs induced AA may be due to treatment by antibiotics (chloramphenicol), nonsteroidal anti-inflammatory drugs, antiplatelet agent (ticlopidine), anticonvulsants (hydantoin compounds, valproic acid, and carbamazepine), gold salts or other drugs.

Chloramphenicol has the best-known association with aplastic anemia. Chloramphenicol metabolites produced by intestinal bacteria are predominantly responsible for marrow aplasia. Reversible agranulocytosis is observed in 2.4 % of patients that have a therapy with ticlopidine (antiplatelet agent used following cerebrovascular accidents and myocardial ischemia); aplastic anemia is much less frequent. These events are characteristically observed in the first 12 weeks of therapy. Anticonvulsants are the drugs most commonly implicated in blood dyscrasias. Gold salts, used in the management of advanced rheumatoid arthritis, were associated with 1.6 aplastic anemia-related deaths per 10,000 prescriptions. The mechanism of aplasia is unclear. A variety of other drugs, including oral hypoglycemic drugs, neuroleptics (particularly phenothiazines), antithyroid agents, and diuretics, have been associated with aplastic anemia.

The association between industrial hydrocarbons such as benzene (and its metabolites particularly hydroxyquinone phenols) and pancytopenia or aplastic anemia is well established. Organochloride and organophosphate pesticides are associated with aplastic anemia based on epidemiologic data. The mechanism of aplastic anemia is unclear.

Deposition of crystalline arsenic in marrow has direct toxicity toward hematopoietic progenitors/precursors resulting in aplasia.

3. *Viral infections*

Variety of viral infections, including hepatitis B, parvovirus B_{19}, and Epstein-Barr virus are associated with aplastic anemia. Some investigators have suggested that antiviral therapy should be considered early in the treatment of aplastic anemia.

4. *Pregnancy*

Pregnancy associated aplastic anemia is a rare association. The relation between these two conditions is still unclear.

Idiopathic Aplastic Anemia

Despite efforts to identify an etiology, approximately 50 % of cases of aplastic anemia appear to be of idiopathic origin. The success immunosuppressive therapy in managing of these patients suggests that immunologic mechanisms may be involved in a majority of these cases.

The basic mechanisms of AA pathogenesis are:

- inherited (congenital) or acquired defect of pluripotent stem cells;
- stromal microenvironment change that leads to disruption of the development and maturation of hematopoietic stem cells;
- insufficient production of hematopoietic growth factors;
- cellular and / or humoral immune suppression of hematopoietic stem cells;
- progressive humoral violation of reparations of the chromosomes telomeres (due to mutations in telomerase).

Clinical manifestations

The clinical picture is due to the development of pancytopenia and consists of three main syndromes: anemic, haemorrhagic and infection.Anemic syndrome is characterized by pallor of the skin and visible mucous membranes, fatigue, weakness, tachycardia, shortness of breath. Hemorrhagic syndrome characterized by spotty-petechial type of bleeding (hemorrhagic rash on the skin, oral mucosa, nasal and gingival bleeding, etc.). Bacterial and fungal infections develop as a result of neutropenia (tonsillitis, pneumonia, sepsis).

Congenital forms of AA are accompanied by congenital malformations and abnormalities of various organs and systems (strabismus, polydactyly, and others.).

Peripheral blood smear: pancytopenia (severe anemia, Hb — 20–80 g/l; normohromia, macrocytosis, decreased reticulocyte count, leukopenia, absolute neutropenia, relative lymphocytosis, thrombocytopenia, increased ESR up to 30–50 mm/h);

Bone marrow examination: reducing the amount of hematopoietic tissue, bone marrow substitution by adipose tissue.

Biochemical analysis of blood: increase in serum iron, ferritin, decrease of TIBC and total protein.

At Fanconi anemia increased the levels of fetal hemoglobin in venous blood.

METAPLASTIC ANEMIAS

This pathology occurs when the proliferation in the bone marrow cells that are not related to erythropoiesis (acute leukemia, multiple myeloma, myelofibrosis, osteomyelosclerosis, tumor metastasis). Blood picture is determined by the underlying disease.

HEMOLYTIC ANEMIAS

Hemolytic anemias is a group anemia caused by hereditary or acquired, a common feature of which is the shortening of the RBCs life. There are a persistent (chronic HA) or massive (acute HA) erythrocyte destruction predominance of their formation. The disease is manifested by syndromes of enhanced hemolysis and compensatory gain of erythropoiesis.

Hemolytic anemias have the following features:

- premature destruction of red cells and a shortened red cell life;
- accumulation of hemoglobin degradation products;
- compensatory increase in erythropoiesis and elevated erythropoietin levels.

Classification of hemolytic anemias:

I. Heredity hemolytic anemia:

1. Membranopathias (hereditary sherocytosis)

2. Enzymopathias (G-6-PD deficiency)

3. Hemoglobinopathias:

- Qualitative — abnormality of primary structure (sickle cell anemia).
- Quantative — impairment of synthesis or absence of one of the globin chains in case of intact primary structure (thalassemia).

II. Acquired hemolytic anemia:

1. Immune hemolytic anemias:

- *Iso (allo) immune* (transfusion of incompatible blood, hemolytic disease of newborn.
- *Heteroimmune* (virus, bacterial infections, chemical, drug-induced).
- *Autoimmune hemolytic anemia.*

2. Nonimmune hemolytic anemias:

- *Toxico-hemolytic:*

— mushroom and snake venoms;
— plumbum, arsenicum, phenylhydrazine;
— endotoxins (burns, uremia, cord. liver).

- *Infectious* (bacterial, parasitic (malaria)).
- *Mechanical*:

— sudden spasm of blood vessels;
— prosthetic heart valves, vascular;
— hypersplenism.

In hemolytic anemia hemolysis of RBCs may occur extravascular (intracellularly as well as the physiological hemolysis), or directly into the blood vessels (intravascular).

Hemolysis is defined as shortened red cell survival, may result from any number of intrinsic or extrinsic abnormalities.

Etiology of hemolysis:

All reasons may be distinguished into to groups: intrinsic and extrinsic abnormalities. Intrinsic abnormalities of red cell defects include membrane defects, ab-

normal hemoglobins, and enzyme defects. Extrinsic abnormalities include microangiopathy, mechanical heart valve, anti-RBC antibody, toxins, and extreme heat.

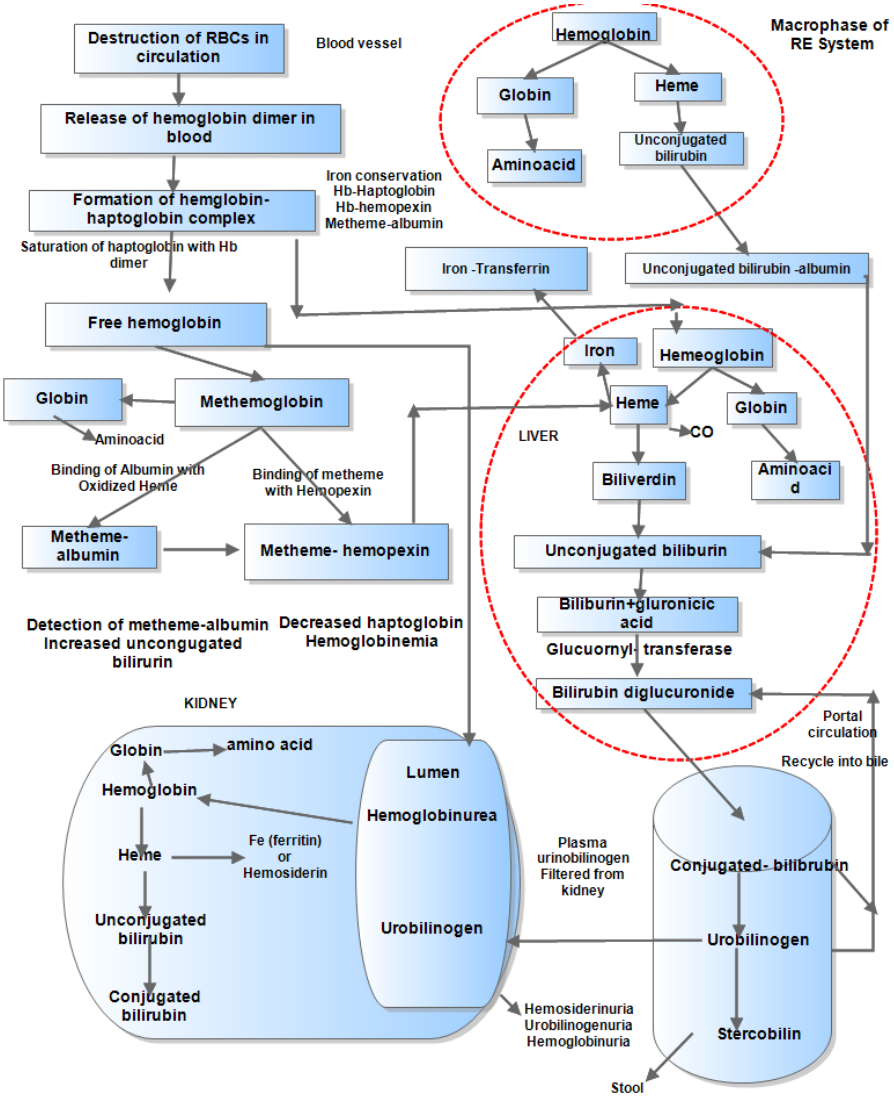


Figure 5 — Scheme of hemolysis

Intracellular (extravascular) hemolysis

Extravascular hemolysis is a results from phagocytosis of intact (but abnormal) RBCs by macrophages in the spleen, liver, and bone marrow. Reduced deformability makes passage through the splenic sinusoids difficult, leading to red cell sequestration and phagocytosis. In the RES (bone marrow, spleen, or liver), proteases convert globin into amino acids, heme protoporphyrin is oxidized by heme oxidase to biliverdin, reduced by biliverdin reductase to indirect bilirubin. Indirect bilirubin is conjugated to form bilirubin diglucuronide (direct bilirubin) by the liver and normally excreted in the bile. In the small bowel, gut bacteria reduce bilirubin to urobilinogen. A small amount of urobilinogen is absorbed by the small bowel and excreted in the urine. Heme iron is transferred to plasma apotransferrin for transport to tissues, most prominently to the bone marrow.

Intravascular hemolysis

Intravascular hemolysis is caused by destruction of circulating RBCs by extreme heat, toxins, infectious agents, intracellular parasites (e.g., falciparum malaria), complement, mechanical damage by artificial vessels, heart valve, and changes the RBCs resistance, membrane permeability and integrity. As a result the red cell destabilizes to osmotic gradient, leading to rapid influx of sodium and water, cell swelling, and physical disintegration. The large amounts of free hemoglobin released from lysed red cells are promptly bound by haptoglobin, producing a complex that is rapidly cleared by mononuclear phagocytes for degradation to peptides (from globin), iron, and bilirubin (from heme). Hemoglobin binding capacity of haptoglobin is 100 mg% (100 mg of hemoglobin per 100 ml of blood). As serum haptoglobin is depleted, free hemoglobin oxidizes to methemoglobin (brown color). The renal proximal tubular cells reabsorb and catabolize much of the filtered hemoglobin and methemoglobin, but some passes out in the urine (imparting a red-brown color). Iron released from hemoglobin can accumulate within tubular cells, giving rise to renal hemosiderosis.

Table 10 — Distinctive features of intravascular hemolysis and intracellular

Signs of hemolysis	**Types of hemolysis**	
	intravascular	**intracellular**
Localization of hemolysis	Vessels	RES
Localization of hemosiderosis	Tubules of the kidneys	Spleen, liver, bone marrow
Yellowness of the skin and mucous membranes	Moderate	Severe
Enlarged liver and spleen	Slight	Significant
Leading laboratory signs	Normochromic anemia, reticulocytosis, hypersideremia, erythroid hyperplasia in the bone marrow	
	Hemoglobinemia Methemoalbuminemia Hemoglobinuria Hemosiderynuria Indirect hyperbilirubinemia Absence or reduced of free serum haptoglobin	Elevated stercobilinogen in feces and urine urobilinogen indirect hyperbilirubinemia increased excretion of bilirubin by bile

Hemolysis can occur continuously (chronic course) or occasionally in the form of crises resulting from a sharp increase of erythrocytes degradation via the action of provocative agent. Depending on the pathogenesis and clinical course there are several types of crisises: hemolytic, aregenerative, sequestration and painful.

Hemolytic (hyperregenerative) crisis is a classic manifestation of acute hemolytic anemia in action of provocative factor (infection, cold, drugs, chemicals, hypoxia and others.). Clinical and laboratory is characterized by anemia, hyperreticulocytosis, hyperbilirubinemia and jaundice.

Aregenerative (aplastic) crisis is a consequence of defect compensatory-adaptive capabilities of hematopoiesis and violations of production of erythroid

cells in BM. Most often occurs when an infection caused by parvovirus B_{19}. Appears anemic syndrome, may be accompanied by hepatomegaly, splenomegaly. Number of reticulocytes in peripheral blood strongly reduced.

Sequestration crisis is rare. It is based on intravascular hemolysis. Primary place of RBC destruction are the spleen vessels, rarer — liver. Along the symptoms of hemolysis (jaundice, anemia syndrome) appear sudden pain in the spleen and liver, abdominal pain, nausea, splenomegaly, hepatomegaly, hypovolemic shock (due to discharge a large amount of blood in the spleen).

Vasoocclusive (painful) crisis is common in qualitative hemoglobinopathies. Aggravating factors are dehydration, infections, hypothermia. By clinical hemolysis is attached pain resulting from vascular occlusion (eg, by sickle cells). The most characteristic pain is musculoskeletal (myalgia, arthralgia) and abdominal localization.

HEREDITARY MEMBRANOPATHIAS

Hereditary sherocytosis (anemia Minkovsky-Shoffar's)

Hereditary sherocytosis is a hereditary membranopathy with intracellular hemolysis. This disorder is caused by intrinsic defects in the red cell membrane skeleton that made red cells spheroid, less deformable, and vulnerable to splenic sequestration and destruction. In about 75 % of cases is an autosomal dominant inheritance pattern.

Pathogenesis

Membrane protein defects are mutation in ankyrin (the most common) and mutation in band 2, spectrin (α and β) or band 3 account. Due to membrane defect and dysfunctional Na^+/K^+-ATPase pump the results it is an increasing permeability of RBC to sodium and spherocytes formation. In spleen spherocytes lose part of erythrocytes membrane and turn into microspherocytes. Life time of spherocytes decreases until 8–12 days.

Clinical manifestations:

- intracellular hemolysis lead to splenomegaly;
- jaundice due to increased unconjugated bilirubin;
- due to increased concentration of conjugated bilirubin in bile increased incidence of calcium bilirubinate gallstones.

There are three variants of the clinical course: mild, medium and heavy. Manifested in mild compensated hemolytic anemia; patients do not need blood transfusions. Hereditary spherocytosis moderate severity is the most common manifestation of the disease and is characterized by moderate anemia, splenomegaly and the need for transfusion only during hemolytic crisis. Severe form is rare (3 % of patients) and shows a heavy anemia (require regular substitution hemotherapy), severe splenomegaly and development of aplastic crises. The

children with severe form is revealed specific skeletal deformities (stigmas of disembryogenesis): a square tower skull; poor dentition; high (gothic) upper palate; syndrome of short little finger. A typical complication is cholelithiasis. A relatively rare complication is shin trophic ulcers.

Peripheral blood smear:

- normochromic normocytic hyperregenerative anemia varying severity: HB out of crisis is 100–110 g/l, during hemolytic crisis up to 40–50 g/l; in 50 % of cases MCHC increased to 38–39 %;
- microspherocytosis, anisocytosis, poikilocytosis, target cells; reticulocytosis out of crisis 3–10 %, immediately after the crisis — more than 10 %;
- WBC out of crisis is normal, during hemolytic crisis — leukocytosis with neutrophilia;
- ESR during the crisis increased.

Bone marrow examination

Bone marrow at spherocytosis hyper- or normocellular predominate red cell line (leuko-erythroblastic ratio is1:2–1:3). Type of hematopoiesis is normoblastic. After the crisis increases the amount of basophilic erythroblasts and normoblasts («blue» bone marrow). In frequent hemolytic crisis appear megaloblasts (due to the rapid consumption of folic acid). At aregenerative crisis changes in bone marrow are the same as in aplastic anemia.

Biochemical analysis of blood: hyperbilirubinemia, increased LDH activity and the concentration of iron, at least — serum ferritin.

Hereditary elliptocytosis

Hereditary elliptocytosis is usually seen as an autosomal dominant disorder affecting primarily people of African descent.

Pathogenesis

It results from defects in the membrane proteins spectrin, protein 4.1 or glycophorin C.

Clinical manifestations

Hereditary elliptocytosis does not usually lead to symptomatic disease. Majority of patients have no anemia or a mild hemolytic anemia and splenomegaly.

Peripheral blood smear:

- elliptocytes virtually 100 % of RBCs in peripheral blood;
- decreased RBC osmotic resistance.

HEREDITARY ENZYMOPATHIES

Hereditary enzymopathies arise due to defect of erythrocytes enzymatic systems:

1) abnormalities of the pentose phosphate shunt: deficiency of glucose-6-phosphate dehydrogenase (the most widespread); deficiency of 6-phosphogluconate dehydrogenase; defect of glutathione synthesis;

2) deficiency of enzymes of glycolysis: deficiency of pyruvate kinase (the most widespread), hexokinase, glucose phosphate isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, phosphoglycerate kinase;

3) deficiency of enzymes of glutathion cycle: glutathione synthetase, glutathione reductase, glutathione peroxidase.

G-6-PD deficiency (glucose-6-phosphate dehydrogenase deficiency)

X-linked recessive disorder.

Pathogenesis

It is a decreasing synthesis of NADPH and glutathione in the pentose phosphate pathway which defends red cell proteins (particularly hemoglobin) against oxidative damage. Proper RBCs function requires only 20 % of the enzyme. Glutathione normally neutralizes hydrogen peroxide, an oxidant product in RBC metabolism. Peroxide oxidizes Hb, which precipitates in the form of Heinz bodies. Heinz bodies are removed in the spleen, leaving erythrocytes with a missing section of cytoplasm (formatted «bite cells»). The altered erythrocytes undergo both intravascular and extravascular destruction.

***Clinical manifestations*:**

Most patients have no clinical or laboratory evidence of ongoing hemolysis until an event — infection, drug reaction (primaquine, chloroquine, dapsone, sulfonamides) or ingestion of fava beans or inspiration of it pollen (mainly in Mediterranean variant). After an oxidant stress, sudden onset with back pain and hemoglobinuria up to 2–3 days.

Clinical forms of G-6-PD deficiency:

- acute intravascular hemolysis;
- favism;
- hemolytic disease of newborn, not associated with the blood group and rhesus;
- hereditary chronic hemolytic anemia (non-spherocytic);
- latent (asymptomatic) form.

Peripheral blood smear:

• normochromic, normocytic hyperregenerative anemia;

• aniso- and poikilocytosis (bite cells), basophilic stippling, Heinz-Ehrlich bodies in erythrocytes,

• leukocytosis with left shift.

Bone marrow examination: hypercellular, reactive hyperplasia of erythroid line in which the proportion of erythroid cells can reach up to 50–70 % of the total number of myelokaryocytes. Type of hematopoiesis is normoblastic. The phenomenon erythrophagocytosis is detected.

Biochemical analysis of blood

• at hemolysis increases the content of unconjugated bilirubin in the serum, but its level rarely reaches very high rates.

• a sharp increase in the concentration of free hemoglobin in the blood and decreased levels of haptoglobin.

Urinalysis: hemosiderin and free hemoglobin are detected in urine.

Pyruvate kinase (PK) deficiency

Autosomal recessive pattern.

Pathogenesis

It is a rare enzyme disorder of the Embden-Meyerhof pathways. RBCs lacking this enzyme are unable to generate ATP from adenosine diphosphate for membrane function. Chronic lack of ATP causes membrane damage. Result is dehydration of the RBC (echinocytes).

Clinical manifestations

Hemolytic anemia with jaundice is begun at birth. Hemolysis was localized intracellularly occurs evenly in different organs containing reticuloendothelial cells. In patients are detected pallor, jaundice and splenomegaly. With age, developed gallstone disease, secondary iron overloads and changes of bones (due to frequent blood transfusions). Aplastic crises triggered by parvovirus B_{19} infection.

Peripheral blood smear:

• normochromic, normocytic hyperregenerative anemia;

• aniso- and poikilocytosis (echinocytes).

Bone marrow examination: normoblastic erythroid hyperplasia. Erythrophagocytosis.

HEREDITARY HEMOGLOBINOPATHIAS

Hemoglobinopathias:

1. Qualitative — abnormality of primary structure of Hb (sickle cell anemia).

2. Quantative — impairment of synthesis or absence of one of the globin chains in case of intact primary structure (thalassemia).

Sickle cell anemia

Sickle cell anemia is autosomal codominant, inherited in simple mendelian fashion.

Pathogenesis

It results from point mutation: replacement of glutation acid (HbßA) by valine (HbßS) in the 6th amino acid of the β chain. Homozygous inheritance re-

sults in sickle cell disease, with most of the hemoglobin being HgßS. Heterozygous inheritance results in sickle cell trait, in which HgßS and Hgß A are present. Red blood cells possessing HgßS as the majority hemoglobin are insoluble or rigid in areas of low oxygen concentration, such as the spleen, liver, kidneys, joints and extremities. Hgß S is structural instability and forms liquid tactoids or polymers of hemoglobin that appear as long, thin bundles of fibers under electron microscopy (sickle cells). Formation of sickle cells is also induced by hypoxia, acidosis, dehydration, fever, and exposure to cold. Sickle cells adhere to vascular endothelium and plug small arterioles, capillaries (leads to infarction) and veins (predisposes to thrombosis). Fragility of sickled RBCs destroys it by mechanical trauma of circulation and causes hemolysis.

Sickle cells may be able to revert to the discoid shape or wheat shape (reversible sickle cell) in to the oxygenated environment of the lung.

Clinical manifestations

Most symptoms occur only in patients who are homozygous.

Main manifestations are:

• chronic hemolytic anemia;
• recurrent painful attacks;
• bacterial infections;
• deterioration of tissue and organ function;
• shortened life expectancy.

Anemia is usually severe but varies highly among patients; mild jaundice and pallor are common. Painful crisis (due to ischemia and infarction) causes severe bone pain usually in low back, also in the tibias and arms, and in joints (result from hemarthrosis or femoral head necrosis). Severe abdominal pain often intractable may develop with or without vomiting must be differentiated from pain due to surgical causes.

Peripheral blood smear:

• normal profile of peripheral blood in HbAS — HbA 55 % to 60 %, HbS 40 % to 45 %; in HbSS — HbS 90 % to 95 %, HbF 5 % to 10 %, no HbA; there are sickle cells and target cells.

• normochromic, normocytic hyperregenerative (reticulocytosis > 5 %), anemia, hemoglobin concentration 60–80 g/l;

• aniso- and poikilocytosis (sickle cells, target cells, ovalocytes, schistocyte), polychromatophilia of erythrocytes, basophilic stippling of RBCs, single normoblasts;

• sickle cell screen: sodium metabisulfite reduces O_2 tension, which induces sickling.

• leukocytosis up to $12–20\times10^{12}$/l, neutrophilia, shift to the left;

• thrombocytosis;

• ESR reduced.

Bone marrow examination: hypercellular, reactive hyperplasia of erythroid line.

Biochemical analysis of blood: hyperbilirubinemia, elevated LDH, increased serum iron and ferritin, hemosiderosis is rare.

Thalassemia

Thalassemia is among the most common inherited disorders of Hb production.

Pathogenesis

By the genetic mechanism thalassemia divided into homozygous and heterozygous. At homozygous form there is a mutation of all thalassemia genes, which lead to a complete blockade of synthesis of the corresponding globin chain. At heterozygous forms along with the gene of thalassemia a healthy pair of this gene, resulting in the synthesis of globin chain is partially blocked. Homozygous thalassemia determines a more severe course of the disease.

Full or partial violation of the synthesis of one of the globin chains leads to violations of erythrocyte hemoglobinization, hemolysis and ineffective erythropoiesis. It results from unbalanced Hb synthesis of at least one globin polypeptide chain (α, β, δ, γ) distinguish α-, β-, δ-, and γ-thalassemia.

Table 11 — Clinical and genetic classification of thalassemias

Clinical syndromes	Genotype	Clinical features	Molecular genetics
β-thalassemias			
β-thalassemia major	Homozygous β-thalassemia (β^0/β^0, β^+/β^+, β^0/β^+)	Severe; requires blood transfusions	Mainly point mutations that lead to defects in the transcription, splicing, or translation of β-globin mRNA
β-thalassemia intermedia	Variable (β^0/β^+, β^+/β^+, β^0/β, β^+/β)	Severe but does not require regular blood transfusions	
β-thalassemia minor	Heterozygous β-thalassemia (β^0/β, β^+/β)	Asymptomatic with mild or absent anemia; red cell abnormalities seen	
α-thalassemias			
Silent carrier	-/α α/α	Asymptomatic; no red cell abnormality	Mainly gene deletions
α-thalassemia trait	-/- α/α (Asian)	Asymptomatic, like β-thalassemia minor	
	-/α -/α (black African, Asian)		
HbH disease	-/- -/α	Severe; resembles β-thalassemia intermedia	
Hydrops fetalis	-/- -/-	Lethal in utero without transfusions	

Full clinical picture of severe hemolytic anemia occurs when homozygous inheritance violations of β-chains synthesis — Cooley's disease. It manifests physical and mental immaturity, pale-icteric skin with signs of hemosiderosis (gives for skin a greenish-brown color), the deformation of the skull bones (tower skull, maxilla enlargement, malocclusion, on X-ray — expansion of the medullary canal of long bones, transverse striations of the skull flat bones — the needle periostosis), leg ulcers, severe hepato- and splenomegaly.

Peripheral blood smear:

• hypochromic microcytic hyperregenerative anemia, the severity of which depends on the form of thalassemia;

• anisocytosis (microcytes), poikilocytosis (target cells);
• hypochromia of RBCs, polychromatophilia, basophilic stippling;
• increase in siderocytes;
• number of WBCs and platelets — within norm.

Bone marrow examination: hypercellular, reactive hyperplasia of erythroid line, sideroblasts content increased.

Biochemical analysis of blood: hyperbilirubinemia, elevated LDH, increased serum iron and ferritin.

ISOIMMUNE HEMOLYTIC ANEMIAS

Hemolytic disease of newborn (HDN)

Acquired HA, resulting antigenic differences of RBCs mother and child, mothers develop antibodies against fetal Ag. The transplacental passage of maternal IgG antibodies through the placenta (e.g., anti-D antibodies, anti-AB antibodies in O mothers) resulting in an extravascular hemolytic anemia in the fetus.

Pathogenesis

The first pregnancy Rh-negative mother with Rh-positive fetus usually proceeds normally. During childbirth occurs maternal immunization by antigens of the fetus RBCs with development of anti-erythrocytic antibodies (anti-Rh(D)-IgG). During her second pregnancy with Rh-positive fetus, antibodies fixed on fetal erythrocytes and cause the death of RNCs by intracellular hemolysis with the development of fetal erythroblastosis.

***Types of HDN*:**

1. Intrauterine fetal death with maceration.
2. Edematous.
3. Anemic.
4. Congenital icteric.
5. Postnatal icteric.

Manifestations

The main symptoms of HDN are jaundice, hepato-splenomegaly and, in severe cases — edema, ascites (due to lack of blood flow). The most dangerous symptom of anemia is «kernicterus» with signs of the nervous system damage due to the toxic effect of indirect bilirubin, which include nystagmus, twitching, high-pitched cry. There are cases of stillbirth.

Transimmune hemolytic anemia

Hemolytic anemia occurs at penetration into the body of newborn erythrocytic antibodies from mother suffering from autoimmune hemolytic anemia.

HETEROIMMUNE HEMOLYTIC ANEMIAS

Heteroimmune hemolytic anemias develop due to formation of antibodies against haptens. These haptens may be drugs (penicillin, sulfonamides and etc.) or viruses.

About 15 % of acquired immune hemolytic anemias are related to drug administration. Some drugs lead to development of autoimmune hemolytic anemia, other as a haptens lead to formation of drug-dependent antibodies that react with RBCs only in the presence of this drug resulting in hemolysis by complement activation.

Table 12 — Drug-induced immune hemolysis

Type of serological reaction	Severetyof hemolysis	Detection of drug-induced antibodies	Drugs
Drug-dependent antibody	Severe often with intravascular hemolysis and renal failure	Serum + drug + RBCs	Quinidine, quinine
Passive agglutination	Moderate severery usually without intravascular hemolysis	Serum + drug-coated RBCs	Penicillins, cephalosporins
Autoantibody	Moderate severery usually without intravascular hemolysis	Serum + RBCs	Methyldopa, procainamide

AUTOIMMUNE HEMOLYTIC ANEMIAS

Classification of autoimmune hemolytic anemia:

- With incomplete warm agglutinins:
  - ✓ Idiopathic.
  - ✓ Symptomatic.
- With full cold agglutinins:
  - ✓ Idiopathic cold hemagglutinin disease.
  - ✓ Symptomatic.
- Paroxysmal cold hemoglobinuria (anemia Donath-Landsteiner):
  - ✓ Acute form.
  - ✓ Chronic form.
- Hemolysine:
  - ✓ Acid-hemolysine.
  - ✓ Warm hemolysine.

Types of antibodies:

1. Incomplete warm agglutinins.
2. Full cold agglutinins.
3. Warm hemolysins.
4. 2-phase hemolysins.

AIHA with warm antibodies is diagnosed in 70 % of cases as idiopathic AIHA and as symptomatic (on background of different diseases — Hodgkin's

disease, CLL, systemic lupus erythematosus, at certain medications, such as penicillin). It occurs in all age groups. Heat antibodies often belong to the class IgG (sometimes — to IgA), predominantly directed against antigens of the Rh system and cause hemolysis at 37 °C. The leading mechanism for implementing the immune effect is the interaction of anti-erythrocytic antibodies with Fc-receptor of phagocytic cells and the subsequent development of intracellular hemolysis.

AIHA with warm antibodies can have both acute and gradual onset. Patients with acute onset have rapidly increasing weakness, shortness of breath, sometimes pain in the heart, fever, vomiting, severe pallor, intense jaundice. At the gradual beginning appears arthralgia and subfebrile temperature, slow increase in anemia, weakness, jaundice. The majority of patients have splenomegaly, hepatomegaly and related calculous cholecystitis.

AIHA with cold antibodies found in 20 % of cases. Often affects the elderly people. In childhood is observed as a symptomatic form, complicating the course of acute Mycoplasma pneumonia, infectious mononucleosis and systemic connective tissue diseases. Interaction of antigens with RBCs occurs when the temperature drops below 37 °C. The most active binding of cold antibodies to the erythrocytes is observed in the temperature range +4–+15°C. Cold antibodies cause predominantly intra-vascular hemolysis, with the participation of the complement system.

AIHA with cold antibodies begins gradually. Patients complain of weakness, malaise, decreased performance, cold intolerance. At low temperatures, observed acrocyanosis (blue, then paleness of fingers, toes, ears, nose); there is a sharp pain in the limbs. With a significant supercooling occurs typical Raynaud's syndrome. Hemolysis has predominantly intravascular character, so it is possible appearance of dark-colored urine due to hemoglobinuria and hemosiderinuria. The liver and spleen are usually not enlarged. The course of disease is chronic. Hemolytic crises are rare.

AIHA with biphasic Donath-Landsteiner antibodies is rare and accounts for about 2 % of all AIHA. Etiologic factor often is hypothermia, viral infection, congenital syphilis. Donath-Landsteiner antibodies belong to the class IgG and directed toward the erythrocyte P-antigen. Binding of biphasic antibodies to erythrocytes occurs at temperature of 0–15 °C (cold phase), but hemolysis occurs only at a temperature of +37 °C when the resultant antigen-antibody complex fix complement. Hemolysis is intravascular character.

Paroxysmal cold hemoglobinuria is an example of AIHA with biphasic antibodies. The clinical picture develops within a few hours after hypothermia and is characterized by fever, chills, pain in the abdomen or in the lumbar region, nausea, vomiting and appearance of dark brown or black urine. Most of the symptoms are stopped after a few hours. Excretion of black urine (due to intravascular hemolysis) is lasts for about two days. Spleen may be palpable in the background of hemolytic crisis. There are vascular manifestations: chilliness, acrocyanosis, Raynaud's syndrome.

Peripheral blood in AIHA:

- normochromic, normocytic anemia;
- poikilocytosis of RBCs: schistocyte, single microspherocytes;
- single normoblasts;
- reticulocytosis;
- leukocytosis up to $10–15\times10^9$/l with a shift of leukocyte formula to the left;
- increased ESR (30 mm/h);
- platelet count is normal or reduced.

Bone marrow

Red lineage sharply expanded. Type of hematopoiesis is normoblastic, sometimes with megaloblastic features due to folic acid deficiency, heavily consumed during hemolytic crisis.

Biochemical blood analysis: hyperbilirubinemia, increased LDH, reduced haptoglobin. The content of serum iron increased or upper limit of normal.

NON-IMMUNE HEMOLYTIC ANEMIAS

The main reasons for non-immune hemolytic anemias:

1. Infectious agents: intracellular parasites (Plasmodium falciparum, Bartonella), bacterias (meningococcus, pneumococcus, Gram-negative bacteria) cause microangiopathic hemolysis.

2. Chemical and physical factors: drugs, industrial chemicals, high body temperature (including — in burn disease).

3. The mechanical lysis of erythrocytes: DIC syndrome, vasculitis, vascular and intracardiac prostheses.

4. Acquired erythrocyte membrane damage: liver disease, paroxysmal nocturnal hemoglobinuria.

Non-immune hemolytic anemia is characterized by the combination of clinical and laboratory signs of intracellular and intravascular hemolysis. Coombs test negative. For adequate treatment is necessary to remove the causes or to stop contact with the substance, that caused hemolysis. With the development of renal failure, hemodialysis is shown.

ERYTHROCYTOSIS

Erythrocytosis — is an increase in the content of red blood cells. There are two groups of erythrocytosis: relative (increase in red blood cells and hemoglobin in a unit volume of blood without increasing their absolute number) and absolute (absolute increasing the number of red blood cells).

Relative erythrocytosis is divided into:

- ***hemoconcentration*** — occur with a decrease in plasma volume (hemoconcentration) due to dehydration (with uncontrollable vomiting, diarrhea, sweating, burn patients, etc.);

• ***stress-erythrocytosis*** — develop due to the «release» of the erythrocytes from blood-pool (as the stress response in vascular-reflex phase of compensatory reactions in acute blood loss, Gaisbock's syndrome (spurious polycythaemia smokers), hypertension, etc.).

Absolute erythrocytosis are due to an increase in erythropoietic bone marrow function, with an increased production of erythropoietin in the body are:

• ***hypoxic*** — develop as a result of increased production of erythropoietin by cells of kidney juxtaglomerular apparatus in response to long-term hypoxia: the decrease in partial pressure of oxygen in the air (in people who have caisson work at mountainous disease, etc.), and respiratory diseases (asthma, emphysema, interstitial pneumonia, diffuse pneumosclerosis, etc.), pathology of the cardiovascular system (heart disease, hypertrophic cardiomyopathy, hemorrhagic telangiectasia, etc.), local ischemia of the kidneys (renal cysts, hydronephrosis, renal vascular damage, etc.);

• ***tumor*** — developed through the production of erythropoietin by tumor cells: in pheochromocytoma, hypernephroma, hepatocellular carcinoma, gastric carcinoma, and others.

Production of erythropoietin by juxtaglomerular apparatus of the kidneys is normal — erythremia (or polycythemia vera) arising from myeloproliferation due to tumor erythroid hyperplasia in a defect of precursor cells of myelopoiesis.

The group of the absolute erythrocytosis also includes endocrine erythrocytosis arising from the ability of a number of hormones have a direct or indirect (via increased production of erythropoietin by juxtaglomerular apparatus of kidney cells), stimulating effect on erythropoiesis: with hyperthyroidism, Cushing's syndrome, hyperaldosteronism, hyperandrogenemia, and others. Hereditary (family) erythrocytosis described.

CHAPTER 4
PATHOPHYSIOLOGY OF LEUKOCYTES

LEUKOPOIESIS

Neutrophiles

The process of formation the mature neutrophil from the myeloblasts carried out in BM within 10-13 days. Can be represented schematically: myeloblast → promyelocyte → myelocyte → metamyelocyte → band cell → segmented neutrophil. At elevated body's needs, this process can be considerably accelerated. After maturation band and segmented neutrophils do not leave BM immediately, and stored in the sinuses for 4–5 days before entering the bloodstream. These cells make up the so-called bone marrow granulocyte reserve, which is the number of almost 30 times exceeds the number of circulating leukocytes. Cells of

bone marrow reserve every 3–5 days updated. From the usefulness of bone marrow reserve the outcome of the disease often depends. The total time of circulating mature neutrophils is 6–12 hours, after which the cells were transferred into tissue, where they live 4–5 days. Neutrophils, past the endothelial barrier between blood and tissues, do not return to the circulation. Within 2–4 days, they die by apoptosis, while in case of inflammation — by necrosis.

Eosinophils

Duration of eosinophils maturation is approximately 3–4 days. After this period they are stay in BM 2–5 days, and then go into the bloodstream, where circulate 10–18 h, then migrate into tissues and accumulate in the largest amount in the skin, lungs, gastrointestinal tract. Their tissues lifetime is up to 6 days. It is believed that tissue eosinophils can be returned to the bloodstream, which explains the length of eosinophilic reactions.

Basophils

Differentiation of basophils in BM lasts up to 5 days. Basophils found in the bloodstream approximately 10h, then arrive in tissues where after 1-2 days after realization of basic effector function they die.

Monocytes

In the process of monocytes differentiation and take place following stages: pluripotent hematopoietic stem cells → pluripotent hematopoietic progenitor myelopoiesis cells → unipotent progenitor granulo-monocytopoiesis cells → monocyte progenitor cells → monoblasts → pro-monocytes → monocytes.

The maturation process of monoblasts in monocytes occurs in BM approximately 5 days, after which the cells out in the bloodstream unlike granulocytes without forming bone marrow reserve. Monocytes are located in peripheral blood from 36 to 104 hours, and then they penetrate into tissue, where transformed into macrophages. Extravascular pool of monocytes is approximately 25 times greater than intravascular. Lifespan tissue macrophages — up to several months. They can be returned to the bloodstream. In these cases, blood smears can be found original monocytoid cells — histiocytes. Single histiocytes are in the blood of healthy people and in larger amounts (2–3 %), they appear in severe intoxication, protracted septic endocarditis.

Lymphocytes

Lymphocytes with myeloid elements have common pluripotent hematopoietic stem cells, but then they get an independent lineage. In this case, cells maturation undergo following steps: lymphoblast → pro lymphocyte → lymphocyte.

T-lymphocytes

The early T-lymphocyte precursors formed in the BM. Precursors of T lymphocytes leave the bone marrow and migrate to the thymus. Under the influ-

ence of thymic epithelial cells and growth factors produced by them, T-lymphocyte pass a number of consecutive stages of maturation moving from the capsular zone in the cortical and then into the medullary.

Past intrathymic differentiation the T cells out of the thymus through the blood vessels and directed to the periphery. They occupy certain areas of peripheral lymphoid system.

B-lymphocytes

Lymphopoiesis of B lymphocytes in the bone marrow involves five main stages: pluripotent hematopoietic stem cells → common lymphoid progenitors for T and B cells → committed progenitors of B lymphocyte (pro-B cell) → pre-B-cell → immature B cell (the final form of the B cells differentiation in the bone marrow). «Naive» B lymphocytes migrate through the blood vessels in the peripheral lymphoid organs.

Described above stages of T and B lymphocyte maturation is called antigen-independent, as used for the acquisition the ability of cells to recognize antigen. The next step is antigen-dependent (immunogenesis). It occurs in the peripheral lymphoid organs after a meeting with an appropriate lymphocyte antigen.

Through the step of activated lymphocyte, T cells formed regulators and effectors of the immune response. At stimulation of B-lymphocytes there is their transformation into plasma cells (through the stages: activated lymphocyte → plasmoblast → proplasmocyte →plasma cell) and begins an active antibody synthesis.

CHANGES IN THE WBC SYSTEM

Amount of leucocytes in unit of blood volume is $4{,}0–9{,}0\times10^9$/l in a norm. Changes in the WBC system may occur:

1) qualitatively (or functional);

2) quantitatively:

- reactive (temporary) (leukocytosis, leukopenia, leukemoid reactions);
- the nature of the tumor (leukemia, lymphoma).

3) in the character of leukocyte formula.

QUALITATIVE CHANGES OF WBC

Qualitative defects of leukocyte can be formed not only with changes on the number of leukocytes but also carry autonomous character.

Production of pathologic leukocytes may arise as a result of:

- tumorous transformation of leukopoietic tissue in leukemia;
- metabolic disturbances in leukocytes;
- genetically determined structural disturbances (dominant hereditary Pelger anomaly of granulocytes, deficiency of myeloperoxidase, glucose-6-phosphatedehydrogenase G-6-PDG).

Table 13 — Characteristics of pathological changes of leukocytes

Pathological changes of WBCs	Characteristics
Anisocytosis	Decrease (microform) and increase in the size (macropolycytes - giant white blood cells)
Pathology of the nucleus:	
Hypersegmentation	Increasing the number of segments in the nuclei of neutrophils (more than 5 at norm 2–5) or eosinophils (more than 3 at norm 2–3) is a characteristic finding in megaloblastic anemia (b_{12} and folate deficiency), but can also be seen as an inherited autosomal dominant trait (hereditary hypersegmentation of neutrophils)
Hyposegmentation	Reducing the number of segments or a complete lack of segmentation (nucleus can be round, elliptical, in form of bean, peanut, gym weights, pince). Can be inherited (pelger-huët anomaly), or acquired (pseudo-pelger-huët cells) in patients with malignant myeloproliferative disorders and infections or tumors which have metastasized to the bone marrow
Pycnosis	Compaction of chromatin
Rrhexis	Decay of a nucleus into separate parts, the disappearance of intersegmental «threads» in mature granulocytes
Fragmentation	Formation of fragments of nuclear chromatin (micronuclei)
Lysis	Dissolution of the nuclear membrane
Chromatinolysis	Liquefaction of chromatin
Vacuolization	Discolored spots («holes») in the chromatin
Bare nuclei of lymphocytes	Lymphocytes without cytoplasm
Reed-Sternberg giant cell	Giant multinucleated or binuclear cell with prominent eosinophilic inclusion-like nucleoli in patients with Hodgkin's disease
Botkin – Gumprecht shadows (basket cells)	Crushed core of lymphocytes in patients with lymphoproliferative syndromes and particularly in CLL
Pathology of the cytoplasm:	
«Exhaustion» of granularity	Deficiency or absence of specific granules
Toxigenic granularity	Small dark-blue or large rough basophilic granules of methmyelocytes, bands and segmented neutrophils during inflammatory states, burns, and trauma
Azurophilic granularity	Multiple, overlapping nuclei of cells or single large azurophilic granules in the cytoplasm of mature leukocytes
Vacuolization	Discolored spots («holes») in the cytoplasm
Döhle bodies	Round or oval amorphous blue inclusion appear in the neutrophils, bands, and metamyelocytes of patients with infection, burns, uncomplicated pregnancy, toxic states
Auer rods	Cherry color needle- or rod-shaped structures (agglomerated azurophilic grain) occur in the cytoplasm of immature leukocytes (blasts) and more mature cells in some patients with AML
Alder-Reilly granules	Large, coarse, dark purple, azurophilic granules, found in in patients with mucopolysaccharidoses
Chédiak-Higashi granules	Very large red or blue granules that appear in the cytoplasm of granulocytes, lymphocytes, or monocytes in patients with the Chédiak-Steinbrinck-Higashi syndrome
May-Hegglin anomaly	Neutrophils contain small basophilic cytoplasmic granules which represent aggregated ribosomes
Atypical lymphocyte (Downey cells)	Large lymphocytes that contain a greater amount of cytoplasm and bluish tinge of cytoplasm are found in viral illnesses such as infectious mononucleosis

Functional defects in leukocyte

Violations of the functional properties of leukocytes can be hereditary or acquired. They are associated with defects in neutrophil granulocytes due to violation of their margination, adhesion, migration, and microbicidal properties.

Violations of neutrophil function:

1) Hereditary/congenital:

• Violation of adhesion:

✓ absence or reduction in the level expression of β_2-integrins on neutrophils;

✓ lack of E-selectin on neutrophils;

✓ actin dysfunction of neutrophil;

• Violation of chemotaxis:

✓ hyperimmunoglobulinemia E;

✓ defects in the membrane of neutrophils (a syndrome of «lazy» leukocytes);

✓ defects in the complement system;

• Violation of bactericidal activity:

✓ deficit of specific granules in neutrophils;

✓ NADPH oxidase defect;

✓ myeloperoxidase deficit in neutrophils;

✓ lactase deficit in neutrophils;

✓ deficit of glutathione peroxidase, glutathione reductase, glutathione synthetase in neutrophils;

✓glucose-6 phosphatase deficit in neutrophils;

2) Acquired:

• Defects of margination, adhesion, chemotaxis, bactericidal activity of neutrophils;

• Reduction of opsonic activity of plasma.

Disorder of leukocyte maturation

Disorder of leukocyte maturation is caused by the blockade of differentiation at different levels of cell development. Differentiation is provided by certain metabolytes and genetic regulation. This disorder can be associated with a decrease of leukocyte production. The reasons of maturation disorders are:

• genetic defects;

• effect of exo- and endogenic factors (agents of purulent and viral infections, medicinal allergens and intoxication);

• tumorous hyperplasia and leukemoid reaction;

• disorder of the bone marrow barrier and its permeability (influence of CSF, glucocorticoids, interleukins, bacyerial toxins), when immature leukocytes leave the bone marrow.

QUANTITATIVE CHANGES OF WBC

Leukocytosis

Leukocytosis is increase in total amount of WBC in a unit of blood above the upper limit of age norm (9×10^9/l).

Etiology

There are physiological and pathological leukocytoses.

Physiological leukocytosis is characterized by a slight increase in the number of cells per unit volume, short-term and no change in leukocyte formula. Physiological leukocytosis can be due to digestive process, myogenic, emotional leukocytosis, leukocytosis during pregnancy and neonates, at water loss (sweating, etc.).

Causes of pathological leukocytosis are:

- physical — small doses of radiation, traumatic etc.;
- chemical — alcohol, certain medications, hypoxemia, acidosis;
- biological — viruses, bacteria, rickettsiae, parasites; antigen-antibody complexes; increase in BAS (leukopoietins, lymphokines, histamine, decay products of nucleic acids).

Mechanisms of leukocytosis

Mechanisms of leukocytosis can be:

- ***increase in normal leucopoiesis***.

It is reactive type of leukopoiesis activation. It is a proliferation of the leukopoietin-sensitive cells of the bone marrow, which results in leukocytosis. It can be caused by increased production of humoral activators (CSF, interleukins) or decreased production of their inhibitors (keilons, prostoglandins E, lactoferrin and isoferritin). The type of proliferated leukocytes depends on etiological factor).

- ***increase in formation of tumor transformed WBC.***

It is the development of leukemia — leukocytosis is the result of increasing the number of dividing malignant and atypical modified cells and their release from the bone marrow into the bloodstream.

- ***redistribution of WBC in vessels.***

At muscular exercises (myogenic), shock (traumatic, blood transfusion, anaphylactic, etc.), stress, digestion, pregnancy, newborn.)

- ***hemoconcentration.***

It is a consequence of hypohydration in various origins (diarrhea, repeated vomiting, polyuria, hyperventilation, etc.). In such cases, there is an increase in the number of WBC and other blood cells).

Increase in certain type of WBCs:

Neutrophilia

Neutrophilic leukocytosis (neutrophilia) — increase of over 70 % of neutrophils in the hemogram. It can be observed at:

- physiological conditions (stress, physical load, overheating, undercooling, the last trimester of pregnancy);
- inflammation or tissue necrosis (surgery, burns, myocardial infarction, pneumonia, rheumatism, rheumatoid arthritis);
- infections caused by Gr^+ and Gr^- microflora;
- hematologic disorders (acute bleeding, hemolysis, myelo-proliferative disease, myeloid leukemia);
- drugs and BAS (adrenalin, steroid hormones, histamine, heparin);
- metabolic disorders (diabetic ketoacidosis, eclampsia, gout, thyrotoxic crisis);
- tumor growth (in the liver, gastrointestinal tract, bone marrow);
- hereditary neutrophilia.

Practical importance has determining the degree of nuclear shift in leukocyte formula. Nuclear shift index (NSI) is calculated as:

$$\text{NSI} = \frac{\text{promyelocytes (\%)} + \text{myelocytes (\%)} + \text{metamyelocytes (\%)} + \text{bandcells (\%)}}{\text{segmented cells (\%)}}$$

NSI in healthy man is equals 0,05 up to 0,10. Increase in index means nuclear shift to the left, decrease in index — shift to the right.

On this basis distinguish six kinds of neutrophilic leukocytosis:

1) ***without nuclear shift*** (in acute blood loss, stress reactions) — the increase in the total number of neutrophils (> 70 %);

2) ***hyporegenerative nuclear shift to the left*** (in mild infections and inflammations) – the increase in the total number of neutrophils (> 70 %) due to segmented (> 65 %) and band cells (> 5 %);

3) ***regenerative nuclear shift to the left*** (in purulent-septic processes) — the increase in the total number of neutrophils (> 70 %) due to segmented (> 65 %), band cells (> 5 %) and metamyelocytes (> 0.5 %);

4) ***hyperregenerative nuclear shift to the left*** (sign of an unfavorable course of infectious and septic diseases) — the increase in the total number of neutrophils (> 70 %) due to segmented, band cells (> 5 %), metamyelocytes (> 0.5 %) and younger cells (myelocytes, promyelocytes, myeloblasts). Aneosinophilia:

5) ***degenerative nuclear shift to the left*** (the indicator of functional activity showed the inhibition of bone marrow, can occur in severe infectious diseases, endogenous intoxication etc.) — the increase in the total number of neutrophils (> 70 %) due to segmented (> 65 %) and band cells (> 5 %); the appearance of neutrophils with signs of degeneration (vacuolization of nucleus and cytoplasm, toxigenic granularity, karyorrhexis and others) in blood;

6) ***degenerative nuclear shift to the right*** (in radiation sickness, pernicious anemia) — the increase in the total number of neutrophils (> 70 %) due to segmented cells (> 65 %) with increasing of hypersegmented-nuclear neutrophils (more than 5 nuclear segments) in blood.

Eosinophilia

When eosinophilia the number of eosinophils in the peripheral blood above $0{,}35\times10^9$/l or more than 5 % in relation numbers, and when hypereosinophilia their number $1{,}5\times10^9$/l. There are three degrees of eosinophilia:

- minor — the content of eosinophils in the blood ranges 15–20 %;
- moderate — eosinophils is 20–50 %;
- severe — cell content greater than 50 %.

The causes of eosinophilia may be the following:

- allergic disorders (asthma, allergic rhinitis, eczema, occupational lung diseases, insect bites, urticaria, eosinophilic angioedema, drug intolerance);
- parasitic infestations (helminthiasis);
- non-parasitic infection (aspergillosis, brucellosis, infectious mononucleosis, scarlet fever);
- malignant neoplasms (cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma);
- leukemias;
- syndromes of eosinophilic infiltrates in the lungs (volatile eosinophilic infiltration (Loffler's syndrome), chronic eosinophilic pneumonia, tropical pulmonary eosinophilia);
- skin lesions (exfoliative dermatitis, psoriasis, pemphigus);
- collagenoses, vasculitis (periarteritis nodosa, rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus);
- immune disorders (reaction «graft-versus-host disease», congenital immunodeficiency syndrome);
- endocrine disorders;
- idiopathic hypereosinophilic syndrome;
- other (cirrhosis, radiation therapy).

Basophilia

Basophilia (more than 1 % basophils in hemogram) — is rare form of leukocytosis. Main causes of basophilia are following:

- myeloproliferative diseases (CML, polycythemia vera, idiopathic myelofibrosis, essential thrombocythemia, basophilic leukemia);
- exposure of ionizing radiation;
- inflammatory diseases (ulcerative colitis);
- viral infections (measles, chicken pox);
- drugs (estrogens);
- hypersensitivity reactions;
- iron deficiency;
- hyperlipidemia.

Monocytosis

Main causes of monocytosis are:

- persistent bacterial and viral infections (tuberculosis, infectious mononucleosis, measles, rubella etc.);
- inflammatory diseases (ulcerative colitis, sprue, collagenoses etc.);
- recovery of infectious disease;
- systemic vasculitis;
- tetrachloroethane intoxication;
- hemoblastosis, malignant tumors (ovarian carcinoma, breast cancer);
- steroid hormone prolonged intake;
- after splenectomy.

Lymphocytosis

Lymphocytosis (more than 45 % lymphocytes in leukocyte formula) may be observed at:

- physiological lymphocytosis (children after 5 days to 5 years, vegetarians and after exercise (myogenic));
- infectious diseases (typhoid fever pertussis, malaria, infectious mononucleosis etc.);
- acute viral infection;
- chronic infectious (brucellosis, tuberculosis, syphilis, etc.);
- malnutrition;
- asthma;
- tumor growth (lympholeukemia, lymphosarcoma);
- endocrine disorders (eunuchoidism, myxedema, acromegaly).

Leukopenia

Leukopenia is decrease in the number of WBC in a unite of blood volume less than lower limit of age norm (4×10^9/l).

Etiology

By origin the leukopenia may be primary (congenital, hereditary) and secondary (acquired). The hereditary leukopenia (usually neutropenia) includes persistent hereditary neutropenia, neutropenia periodic hereditary, genetic monocytopenia (Chediak-Higashi syndrome). The causes of acquired leukopenia are:

- physical (ionizing radiation, X-ray radiation, excessive sun exposure, etc.);
- chemicals (industrial chemicals — benzene, insecticides etc.; drugs — cytostatics, sulfonamides, barbiturates, immunosuppressants; use of food crops affected by fungus; lack of vitamin B_{12} and folic acid);

• biological (hard infections (typhus, virus of influenza, measles, rickettsia toxin, tuberculosis); immune (effect of antibodies against leukocytes); excess of BAS (catecholamines, leukotoxins etc.) in shock, stress; hormonal (stress, re-distributive type of leukopenia).

Mechanisms of leucopenia:

1) Violation or suppression of the WBC formation as a result of:

• violation of proliferation and differentiation of hematopoietic stem cells:

✓ genetic defects of hematopoietic stem cells;

✓ lack of the components needed for leukopoiesis (eg., proteins, phospholipids, amino acids, hypovitaminosis — B_{12}, folic acid, etc.);

✓ disorders of mechanisms of leukopoiesis humoral regulation (hypothyroidism, hypocorticism, reducing leukotrienes or sensitivity to them of the germ cells leukocyte hematopoiesis);

✓ loss of the ability of hematopoietic stem cells to differentiate;

✓ autoimmune mechanisms associated with the formation of anti CFU-GM antibodies and autoreactive T cells;

• damage of stem cells:

✓ cytolytic (due to ionizing radiation, cytostatic drugs, immune factors (antibodies);

✓ antimetabolic — action of agents interfered in the exchange of purine and pyrimidine bases(some antitumor drugs, levomycetin);

✓ idiosyncratic;

• pathology of hemopoiesis microenvironment, hyposecretion by these cells of growth factors (GM-CSF, G-CSF, IL-3, M-CSF, etc.);

• decreasing area of granulocytopoiesis as a result of substitution of hematopoietic tissue in bone marrow by tumor (leukemia and cancer metastasis to the bone marrow), fibrous, bone, adipose tissue.

2) Leukopenia due to increased destruction of WBC:

• under the influence of antibodies — leukoagglutinins that may formatted during blood transfusion, the action of certain medications haptens (sulfonamides, amidopyrine et al.), toxic factors of infectious origin (severe infectious diseases, extensive inflammation), diseases accompanied by an increase in the number of circulating immune complexes in the blood (autoimmune diseases, lymphomas, leukemias etc.);

• premature cell death due to cytogenetic abnormalities (tetraploidy) in some hereditary neutropenias;

• increased destruction of circulating WBCs in the spleen in cases with hypersplenism (collagenoses, cirrhosis, hemolytic anemia, and others.).

3) Leukopenia due to redistribution of leukocytes, are temporary in nature, found at:

• shock (anaphylactic, traumatic, posthemorrhagic, etc.);

• after heavy muscular work (increases WBCs in the capillaries of muscles, intestines, kidneys and lungs and decreases in other organs);

• during the phenomenon of regional state of leukocytes in large areas of vessels (diffuse peritonitis, pleurisy, phlegmon etc.).

4) Increased loss of WBCs in the presence of fistulas in lymphatic vessels and trunks, plasma- and lymphorrhea, burns, purulent processes.

5) Hemodilution leukopenia (rare) is a consequence resulting hypovolemia large volume plasma transfusion, plasma expanders, saline, liquid outlet from tissues into the bloodstream (under hyperaldosteronism, hyperglycemia, hyperalbuminemia).

Neutropenia

Neutropenia is a condition in which the absolute neutrophil count in blood less than $2{,}0\times10^9$/l. Neutropenias can be distinguished by the number of neutrophils and the risk of infection: mild — $1{,}0–2{,}0\times10^9$/l; moderate severity — $0{,}5–1{,}0\times10^9$/l; severe — less than $0{,}5\times10^9$/l. Severe acute neutropenia is a danger for life. The main reasons of neutropenia are:

- oppression of leucopoiesis in bone marrow (radiation, chemotherapy, leukemia, aplastic anemia, atypical granulopoiesis);
- enhanced destruction (splenomegaly, hemodialysis, autoimmune processes);
- gram-positive bacteria infections (typhoid), viruses (influenza, smallpox, hepatitis B, measles, mumps, AIDS), severe protozoal infections (malaria);
- protein starvation;
- side-effect of medical drugs abuse (semisynthetic penicillins, cephalosporins, sulfonamides in combination with trimethoprim, phenothiazines, antithyroid drugs).

Eosinopenia

Eosinopenia is a decrease in count of eosinophil less than $0{,}02\times10^9$/l. The complete absence of eosinophils called aneosinophilia. The causes are:

- stress;
- Itsenko-Cushing disease or injection of corticotropin and corticosteroids;
- athletic overexertion;
- burns, trauma;
- infectious disease (sepsis, dysentery, typhoid fever and acute appendicitis);
- agranulocytosis.

Basopenia

Basopenia is a reducing the number of basophils below $0{,}01\times10^9$/l. Reasons leading to baspenia:

- thyrotoxicosis;
- Cushing's syndrome;
- hemorrhagic syndrome;
- medicines (progesterone);
- agranulocytosis.

Monocytopenia

Monocytopenia is a rare condition. Monocytopenia is a decrease in count of monocyte less than $0{,}09\times10^9$/l. The causes are:

- radiation sickness;
- severe septic conditions;
- acute severe infectious disease.

Lymphopenia

There is a decrease in count of lymphocytes less than $1{,}2\times10^9$/l. The reasons are:

- protein deficiency (fasting);
- bone marrow failure (radiation damage, the use of immunosuppressive drugs);
- hereditary and acquired immunodeficiencies;
- lymphogranulomatosis;
- accelerated death of lymphocytes (in infections caused by lymphotropic viruses: measles, poliomyelitic, human immunodeficiency virus; under the action of cytotoxic drugs; the action of antilymphocytic antibodies (collagenoses);
- loss of lymphocytes (fistulas and drainage of thoracic duct);
- violation of lymphocyte migration (may occur under the stress and hypercortisolism, when there is a transition of their in tissues and enhanced apoptosis of lymphoid cells).

The value of leukopenias: in severe leukopenia there is a decrease the resistance of the organism (mainly anti-infection and anti-tumor). This is due to the leukocytes are involved in the implementation of humoral and cell immunity and phagocytic responses.

Agranulocytosis

It is a clinical hematological syndrome characterized by the excessive decrease in granulocyte count in absolute numbers (neutrophils, eosinophils, basophils). Decreasing of granulocytes $< 0{,}75\times10^9$/l or/ and decreasing total amount of WBC $< 1{,}0\times10^9$/l.

Classification of agranulocytosis

- by clinical course: flash-like, acute, subacute, recurrent, cyclic;
- by origin: congenital, acquired;
- by mechanism: immune, myelotoxic.

Pathogenesis*:*

The basis of myelotoxic agranulocytosis is a depressing effect of drugs and other damaging factors on the proliferative activity of granulocyte elements of the bone marrow, resulting in develops hypoplasia of granulocytopoiesis. The

possibility of severe granulocytopenia development is determined by the total dose of the received drug (arsenic, vincristin, myelosan). Myelotoxic granulocytosis is usually associated with anemia and thrombocytopenia.

The leading role in the pathogenesis of immune (hapten) agranulocytosis has the appearance of antibodies (agglutinins, lysine, etc.), the action of which is directed against one's own granulocytes in the peripheral blood or progenitor cells in the bone marrow. Medical drugs (amidopyrine, phenacetin, anti-tuberculosis drugs, and anti-epileptic drugs) act as haptens, forming complexes with plasma proteins and membranes of leukocytes. Antibodies is produced on the resulting «alien» complex (antigen), is fixed on the surface of cells, causing their destruction. As a rule, at immune agranulocytosis there is a decreased contents only leukocyte.

Clinical manifestations of agranulocytosis: ulcerative necrotic angina (angina agranulocytotica), developing as a result of the suppression of defense reactions (loss of resistance to bacterial flora).

Panmyelophtisis

It was described by P. Erlich in 1888. It is characterized suppression of bone marrow function: erythrocytes, thrombocytes and leukopoiesis. Thus there is a total devastation of the bone marrow: in it punctate can be founded only a few nuclear elements. In the blood are growing irreversible aplastic anemia (hypo-, normo- or hyperchromic character), leukopenia with agranulocytosis and thrombocytopenia.

LEUKEMOID REACTION

Leukemoid reaction is a reactive functional state of the hematopoietic system, lymphatic and immune systems arising in the background of various diseases. Changes in blood look like leukemia. However leukemoid reaction is not transformed into the leukemia, which they resemble and they end after the completion of the basic pathological process.

There is no transformation of normal hemopoietic cell into tumor cell in bone marrow. There is an activation of normal cell proliferation.

Etiology**:** BAS, viruses, bacteria, helminth.

Mechanism**:**

• increase in the number and activity of leukopoietic stimulators;

• increase in stimulators of WBC differentiation;

• decrease in inhibiting factors of cell proliferation.

Table 14 — Differences leukemoid reactions from leukemia

	Leukemoid reactions	**Leukemia**
Etiology	Infectious agents; BAS, activate the output of blood cells from hematopoietic organs; state, leading to increased «consumption» of blood cells; various immunopathological condition	Carcinogens

	Leukemoid reactions	Leukemia
Mechanisms of development	Activation of normal hematopoiesis and entry of excess blood cells into the bloodstream. Suppression of normal hematopoiesis and braking output of blood cells into the bloodstream (cytopenia forms of leukemoid reactions)	Transformation of normal hematopoietic cells into the tumor
Bone marrow	Focal hyperplasia of normal hematopoietic cells (in proliferative leukemoid reactions). Hypoplasia of the hematopoietic tissue (at cytopenia forms of leukemoid reactions)	Generalized hyperplasia of tumor hematopoietic cells. Usually many blasts and immature leukemic cells
Peripheral blood smear	The presence of blasts and immature forms of WBC (in proliferative responses); leukopenia (cytopenia forms of leukemoid reactions); platelets are usually small with normal aggregation, no thrombocytopenia Signs of degeneration of blood cells. No basophilia, no eosinophilia (observed in Marked eosinophilia)	Cytopenia combined with the presence in the blood of leukemic blast cells; hiatus leukemic in AML; signs of cell degeneration are usually absent (observed in B-lymphoid leukemia) Platelets frequently qualitatively abnormal, large, with abnormal aggregation, thrombocytopenia may occur; eosinphilia, basophilia (in CML)

Table 15 — Types of leukemoid reactions and their characteristics

Types of leukemoid reaction	Causes	Peripheral blood smear
1. Myeloid type		
Neutrophilic:		
Pseudoblastic	Outcome of immune agranulocytosis; primary tuberculosis; severe toxic infections (diphteria, tetanus etc.); sepsis	A lot of blast cells
Promyelocytic		A lot of typical promyelocytes
Like chronic myeloid leukemia	Infections (bacterial, viral, fungous); inflammation (chronic vasculitis, dermatitis, podagra, myositis etc.); intoxications (metabolism impairments, endocrine pathologies, uremia); malignant neoplasms (breast cancer, neoplasm in kidney, liver, lungs); lymphogranulomatosis	Neutrophilia with hyperregeneratoric nuclear shift to the left, normal number of eosinophils, basophils, degenerative changes in neutrophils (toxic granulation, karyopyknosis)
Marked eosinophilia	Parasitosis (filariasis, lambliasis, opisthorchiasis and others); allergic reactions (bronchial asthma, allergic reactions, drug-induced allergic reaction); collagenoses (rheumatoid arthritis, nodular periartheritis, scleroderma systematica); Löffler endocarditis; immune deficient state (Wiskott-Aldrich syndrome, IgA deficiency); malignant neoplasms (thyroid gland, stomach, renal cancer, lymphogranulomatosis, Hodgkin's disease, CML); idiopathic hereditary forms	Increased number of eosinophils (> 15 %) and changes in cell morphology (nucleus and cytoplasm vacualization)
2. Monocytic-lymphocytic type		
Like acute lymphoblastic leukemia	Infectious mononucleosis	Lymphocyte number — 20×10^9/l and more, increased number of monocytes, «atypical mononuclears» (> 10 %), neutropenia

Types of leukemoid reaction	Causes	Peripheral blood smear
Acute infectious lymphocytosis	Enteroviral infection caused by Coxsackie virus; cat scratch disease; bacterial infections (whooping-cough, yersiniosis, tuberculosis and others); protozoal invasion (toxoplasmosis, malaria)	Lymphocyte number — 15–100×10^9/l and more, lymphocytosis (> 60 %) without changesin cell morphology, monocytosis
Stress-lymphocytosis	Cardiovascular pathology (cardiovascular collapse, acute heart failure, myocardial infarction, septic shock and others); immediate hypersensitivity reactions; surgical treatment; trauma; epilepsy	Short-lasting leukocytosis up to 5×10^9/l and more
Long-lasting lymphocytosis	Rheumatoid arthritis, malignant tumors (thymoma); chronic inflammatory diseases (sarcoidosis, Wegener's granulomatosis); delayed hypersensitivity reactions; hyposplenism, smoking	Long-lasting lymphocytosis $3{,}8\times10^9$/l and over
Reactive monocytosis	Infectious inflammatory disease (tuberculosis, chronic pyelonephritis, sarcoidosis, sprue); malignant neoplasms (breast and ovarian cancer, lymphogranulomatosis, multiple myeloma)	Increased monocyte number (> $0{,}8\times10^9$/l)

Among leukemoid reactions monocyte-lymphocytic type most important in practical terms is leukemoid reaction with a picture of acute lymphoblastic leukemia in infectious mononucleosis.

Infectious mononucleosis

***Etiology*:** EBV, herpes virus simplex, CMV, rubella virus, hepatitis B, adenoviruses.

***Pathogenesis*:** is spread via saliva. Incubation period is 4–7 weeks. The virus replicates first within epithelial cells in the pharynx and later within B cells (by CD21). The host immune response involves cytotoxic (CD8-positive) T cells against infected B lymphocytes, resulting in enlarged, atypical lymphocytes (Downey cells). In case of T-lymphocyte reactions absence (T-lymphocyte reactions are cytotoxic to virus) uncontrolles B-lymphocyte proliferation takes place and B-cellular lymphoma may develop.

Symptoms usually persist 2–3 weeks. There are:

- fever;
- pharyngitis with edema and adenoidal hypertrophy, tonsillitis;
- lymphadenopathy — tender, bilateral and symmetrical;
- mild to moderate splenomegaly in 50–75 %;
- atypical features include skin rash, hepatitis and encephalitis.

Hematological signs:

- leucocytosis =$12–18\times10^9$/l;
- atypical mononuclear cells;
- EBV-specific antibodies (to viral capsid antigen and to nuclear antigen;
- high titers of heterophile antibodies.

CHAPTER 5
HEMOBLASTOSIS. LEUKEMIA

Hemoblastosis — is a group of malignant tumors of hematopoietic tissue and lymph organs.

According to primary tumor localization hemoblastosis divided into:

- leukemia (primary localization in the bone marrow);
- lymphoma (primary location outside of the bone marrow).

LEUKEMIA

Leukemia — is a tumor originating from hematopoietic cells in the bone marrow, which the basis of development is the uncontrolled growth of cells with a predominance of proliferation over the differentiation and formation the foci of abnormal hematopoiesis in organs and tissues that are normally not involved in hematopoiesis.

General characteristics of leukemia:

- leukemia is characterized by the proliferation of primary atypical cells in the bone marrow suppression with of normal hematopoietic tissue;
- the ability of leukemic cells for invasion and metastasis leads to subsequent dissemination into the peripheral blood, spleen, lymph nodes and other organs;
- leukemias are often characterized by leukocytosis, presence of atypical and immature cells in the peripheral blood (but not in all forms and not in 100 % of cases).

Principles of leukemia classifications

The following principles are given for leukemia classifications:

1) degree of differentiation (maturity) of leukemic cells;
2) histogenesis and cytogenesis of tumor cells;
3) number of blast cells in peripheral blood.

1. <u>According to the degree of differentiation (maturity) of leukemic cells are distinguished:</u>

- acute leukemia — tumor from BM with complete loss of ability of stem blood cells to differentiation. Is characterized by the rapid increase of immature blood cells;
- chronic leukemia — tumor from BM with partial delayed of ability of stem blood cells to differentiation. Is characterized by the excessive production of relatively mature WBC and their accumulation.

The defining characteristic is not the speed of process current; it is the tumor substrate (the main mass of components of the tumor cell). If the main mass of cells is represented blasts it is acute leukemia; in chronic leukemia — the tumor cells are mature and maturing elements.

2. According to the histogenetic characteristics of leukemic cells:

- neoplasms from the cells of lymphoid lineage;
- neoplasms from the cells of myeloid lineage.

3. According to the number of leukocytes and blast cells in the peripheral blood acute leukemias are divided into:

- leukemic — high level of WBC sometimes more than $50–80\times10^9$/l, a large number of blast cells;
- subleukemic — leukocytosis less than in previous type, blast cells are found in small amounts (3–5 %);
- aleukemic — WBC normal, blast cells are not found in the blood;
- leukopenic — WBC below 4×10^9/l, a small number of blast cells.

Etiology of leukemia

Etiology is unclear in most cases of leukemia.

Theories of leukemia occurrence:

- chemical theory;
- radiation theory;
- viral theory;
- genetic theory.

Chemical theory

In experiment: induction of leukemia in animals was made by introducing carcinogens (dimetilbenzantratsen , methylcholanthrene , etc.), metabolites of tryptophan and tyrosine.

The risk of acute leukemia is increased in people with long occupational exposure to benzene and volatile organic solvents (drivers, workers leather and shoe industry, etc.). Exposure to phenylbutazone, arsenic, thorotrast, and chloramphenicaol may be related to the future development of leukemia. In most cases, bone marrow aplasia caused by drug exposure is the initial event, and acute leukemia evolves later. Cytotoxic therapy, especially with alkylating agents such as melphalan, chlorambucil, and cyclophosphamide, increases the risk for leukemia. The risk seems to be related to the total dose of the alkylator agents that are received.

The incidence of acute myeloid leukemia is increased 1.3–2 times in smokers, presumably because of exposure to carcinogens, such as benzene, in tobacco smoke.

Radiation theory

Ionizing radiation is the cause of radiation leukemia in laboratory animals (single (>2 Gr) or chronic exposition). There are data about increase of the number of cases of leukemia in children, exposed to radiation and in patients who underwented X-ray and radioactive isotope treatment. Increased incidence of AML and CML residents of Hiroshima and Nagasaki at the radiologists, patients treated for malignant tumors with high doses of X-rays, yttrium, radium.

Viral theory

Three lymphotropic viruses — human T-cell leukemia virus-1 (HTLV-1), Epstein-Barr virus (EBV), and Kaposi sarcoma herpesvirus/human herpesvirus-8 (KSHV/HHV-8) — have been implicated as causative agents in particular lymphomas. HTLV-1 is associated with adult T-cell leukemia/lymphoma. EBV is found in a subset of Burkitt lymphoma, 30 % to 40 % of Hodgkin lymphoma (HL), many B-cell lymphomas arising in the setting of T-cell immunodeficiency, and rare NK-cell lymphomas. In addition to Kaposi sarcoma, KSHV is uniquely associated with an unusual B-cell lymphoma that presents as a malignant effusion, often in the pleural cavity.

Genetic theory

CLL has dominant and recessive types of inheritance. Several diseases have predisposing to spontaneous chromosome breakage and nondisjunction of somatic or sex chromosomes (Down, Klinefelter, Turner syndrome, Fanconi anemia etc.).

Philadelphia Chromosome. This chromosome appears as a result of deletion of the chromosome of the 22^{nd} pair and translocation of the separated segment to the 9^{th} pair (in 90 % of the patients). The c-abl oncogene at 9q34, coding for the tyrosine protein kinase p145, is translocated to the bcr (breakpoint cluster region) on 22q. This results in a new and abnormal fusion gene, producing bcr/c-abl mRNA. This mRNA synthesizes a potent tyrosine protein kinase p210 unique to chronic myelogenous leukemia. The p210 protein may be instrumental in the genesis of CML.

Translocation of the 8^{th} chromosome segment to the 14^{th} has the same frequency in lymphoma of Berkitt, most likely because of the influence of Epsteine-Barr's virus. In follicular lymphoma can be found mutation t(14;18) with formation of fusion gene resulting from translocation IgH-bcl-2 (IgH — immunoglobulin heavy chain enhancer) that is an inhibitor of apoptosis.

Pathogenesis of leukemia

Stages of leukemia development:

- Initiation.
- Promotion.
- Infiltration.
- Progression.
- Metastasis.

Initiation

Pathogenic factor (radiation, viruses, etc.) acts on hematopoietic stem cells as a result is the mutation. The mutation leads to the transformation of proto-oncogenes to oncogenes and anti-oncogenes inactivation. To initiate tumor

growth, as a rule, require activation of two or more oncogenes in combination with dysfunction of anti-oncogenes. Due to the malignant transformation the hematopoietic cells go out of control of regulatory systems, their division is activated and differentiation is suppressed.

Promotion

Activation and hyperproliferation of leukemic cells under the promoter to form a clone of leukemic cells those are identical to phenotype and genotype (monoclonal stage).

Infiltration

Dissemination of leukemic cells in the bone marrow with inhibition of normal hematopoiesis.

Progression

Instability of the cell genetic apparatus of the tumor substrate leads to qualitative changes: disruption of the chromosomes structure, aneuploidy and derepression of previously active genes. These changes lead to the appearance of new clones of tumor cells that differ in phenotype and genotype (polyclonal stage). Among the new clones (formatted in the organism life and under the influence of therapeutic agents used in chemotherapy) «selected» the most autonomous clones, which leads to a «malignancy» of the disease. In polyclonal stage leukemic cells become resistant to cytotoxic therapy.

Metastasis

Pathological focuses of bone marrow are formed due to the ability of leukemic cells to the invasion, intra- and extravasion, migration by the vascular system, implantation and proliferation in different tissues and organs.

Evidence of tumor nature of leukemia

Tumor nature of leukemia confirmed the presence of the general signs of uniting leukemias and tumors:

- impaired cells ability to differentiate;
- proliferation (violation of realization of apoptosis);
- morphological and metabolic cell anaplasia;
- tumor progression;
- infiltrative growth;
- ability to metastasize;
- cachexia;
- frequent death of the organism.

Features of leukemic cells

Morphological features of leukemic cells

1. Size:
- increased by 2–3 times or reduced to the size of the lymphocyte;
- anisocytosis.

2. Nucleus:
- increased;
- contours deformed;
- coarse chromatin, its amount increased;
- vacuolization and segmentation of nucleus.

3. Nucleoli:
- the number increased to 8 or more;
- the size is increased to 1/3–1/2 of the nucleus, the more nucleoli — the more malignant process.

4. The cytoplasm:
- sharp basophilia;
- vacuolization;
- grains in the cytoplasm (acute leukemia monoblastic);
- azurophilic grain;
- Auer rods-formation in the form of sticks that resemble crystals.

Azurophilic grain and Auer rods are found not in all forms, more often — in AML.

Cytochemistry features of leukemic cells

Blood cells contain various enzymes, fats, and other substances that can be identified by cytochemical means.

The most important cytochemical studies in the study of acute leukemia are myeloperoxidase (MPO), nonspecific esterase (NSE), PAS, and acid phosphatase (AP).

Myeloperoxidase (MPO) — myeloperoxidase is an enzyme located in the granules of myeloid and monocytic cells. Myeloperoxidase is the most important marker distinguishing myeloid from lymphoid blasts and never founded in lymphoid cells.

Nonspecific esterase (NSE) — is an enzyme that founded in large amounts in monocytic cells, in minor concentrations in myeloid or lymphoid cells. NSE is used to identify monocytes.

PAS (periodic acid Schiff stain) — demonstrates glycogen and related mucopolysaccharides. Myeloid or monocytic blasts are typically weakly positive or negative. A granular PAS is characteristic for lymphoblastic leukemia. PAS staining is positive in the erythroblastic leukemias.

Glycogen distribution pattern is important for differentiation of myelo- and lymphoblasts. In AML it has diffuse distribution, in ALL — granular.

Acid phosphatase (AP) — positive reaction in T-cell acute lympholeukemia and extremely positive in acute acute promyelocytic leukemia.

Tartrate-resistant acid phosphatase (TRAP) is one of acid phosphatase isoenzymes, and important diagnostic feature of hairy cell leukemia.

CML is characterized by reduction in myeloperoxidase activity, acid and alkaline phosphatase. Decrease in alkaline phosphatase in neutrophils differs CML from leukemoid reaction myeloid type.

Cytogenetic features of leukemic cells

Cytogenetic chromosomal analysis and DNA ploidy studies are important diagnostic and prognostic factors in evaluating leukemia. Chromosomal abnormalities are detected in 70–80 % of patients with acute leukemia. Many genetic abnormalities typical for specific nosological forms and confirm their clonal origin. This fact is used for diagnostic and differential diagnostic purposes.

Immunophenotypic features of leukemic cells

Immunophenotyping of blasts permit to determine the presence or absence of CD-markers (cluster of differentiation) on blast cells. In their totality can be determined the origin and the degree of differentiation of leukemic clone. Immunophenotyping is use antibodies (usually monoclonal) to various cell surface and cytoplasmic proteins.

Variants of leukemia-associated phenotypes (CD-markers), presented in table 16.

Table 16 — Cases of some leukemias and associated immunophenotype

Type of leukemia	Immunophenotype
Undifferentiated acute myeloblastic (M0)	CD13, CD33, CD34, CD117, HLA-DR
Acute myeloblastic leukemia with maturation (M2)	CD13, CD33, CD117, HLA-DR
Acute myelomonocytic leukemia (M4)	CD13, CD33, CD14, CD15, HLA-DR
Acute erythroid leukemia (M6)	CD13, CD33, CD36, CD71, HLA-DR

Clinical manifestations

The main syndromes in leukemia:

- anemic;
- hemorrhagic;
- infectious;
- metastatic (hyperplastic syndrome);
- intoxication;
- osteo-arthropathic.

Anemic syndrome is developed due to:

- oppression of erythropoiesis;
- supplantation of erythropoiesis;

• short-life of RBC (defect of cells);
• destroyed of RBC and its stem cells by antibodies;
• concurrent uptaking by tumors cells of substrate needed for erythropoiesis.

Hemorrhagic syndrome is developed due to:

• oppression of megakaryocytopoiesis → thrombocytopenia;
• thrombocytopathy;
• destroyed of platelets and its stem cells by antibodies;
• defect in plasmatic factors of hemostasis → coagulation disorders.

Infectious syndrome is developed due to:

• oppression of granulocyto- and lymphopoiesis;
• structural and functional defect of cells for nonspecific resistance (granulocytes, monocytes, NK);
• structural and functional defect of cells for specific immunity (lymphocytes).

Metastatic (hyperplastic syndrome) is a results of metastasis of leukemic cells to the other organs, with proliferation of tumors cells and enlargement of these organs — lymphadenopathy, hepatomegaly, splenomegaly, tonsil and gum hyperplasia). Leukemic infiltration may occur in skin, CNS (neuroleukemia), mammary glands and ovaries.

Intoxication by products of cells disintegration (nucleoproteins) is a result of cell death (norm and leukemic cells). Symptoms: fever, loss of weight, fatigue.

Tumor lysis syndrome — is a group of metabolic complications that can occur after treatment of leukemias (also in lymphomas) and is caused by the breakdown products of dying tumor cells. From destroyed tumor cells release intracellular ions and large amounts of metabolic byproducts (includes potassium, phosphate and nucleic acids) into the systemic circulation. They result in metabolic abnormalities like hyperkalemia, hyperphosphatemia, hyperuricemia, hyperuricosuria, hypocalcemia, and consequent acute uric acid nephropathy and renal failure.

Osteo-arthropathic syndrome is caused by accumulation of cancer cells in the BM. It leads to the increase in pressure in BM and cell death, which manifested by painful of bones, joints.

ACUTE LEUKEMIA

Acute leukemia — is a tumor originating from bone marrow, with complete loss of the ability of hematopoietic cells to differentiate at the level of blasts.

Morphological substrate of the tumor is blast cells (IV class on modern scheme of hematopoiesis).

Acute myelogenous leukemia (AML) is the most common type of acute leukemia in adults, 45 % of all leukemias and 80–90 % of acute leukemias.

Classifications of acute leukemia

Classification of acute leukemias is made by French-American-British (FAB) working hematologists group on the basis morphological and cytochemical characteristics of blast cells.

The French-American-British (FAB) classification of AML:
M0 — Undifferentiated acute myeloblastic.
M1 — Acute myeloblastic leukemia with minimal maturation.
M2 — Acute myeloblastic leukemia with maturation.
M3 — Acute promyelocytic leukemia.
M4 — Acute myelomonocytic leukemia.
M4 eos — Acute myelomonocytic leukemia with eosinophilia.
M5 — Acute monocytic leukemia.
M6 — Acute erythroid leukemia.
M7 — Acute megakaryoblastic leukemia.

Revised WHO classification of acute leukemia incorporates parameters which include morphology, cytochemistry, immunophenotyping, cytogenetics, molecular genetics (which are related to prognosis) and clinical features. The number of blasts necessary for the diagnosis is more than 20 % in bone marrow when compared to 30 % in FAB classification. AML is classified into seven major categories:

- Acute myeloid leukemia with recurrent genetic abnormalities.
- Acute myeloid leukemia with myelodysplasia-related changes.
- Therapy-related myeloid neoplasms.
- Acute myeloid leukemia, not otherwise specified.
- Myeloid sarcoma.
- Myeloid proliferations related to Down syndrome.
- Blastic plasmacytoid dendritic cell neoplasm.

The French-American-British (FAB) classification of ALL includes:

L1 — ALL with microlymphoblasts. Around 25 to 30 % of adult cases and 85 % of childhood cases of ALL are of this subtype. In this type small cells are seen with regular nuclear shape, homogeneous chromatin, small or absent nucleolus, scanty cytoplasm.

L2 — ALL with typical blasts. Around 70 % of adult cases and 14 % of childhood cases are of this type. The cells are large and or varied shapes with irregular nuclear shape, heterogeneous chromatin, large nucleolus.

L3 — ALL with macrolymphoblasts. This is a rarer subtype with only 1 to 2 % cases. In this type the cells are large and uniform with vacuoles (bubble like features) in the cytoplasm overlying the nucleus.

WHO proposed a classification of ALL used the immunophenotypic classification that includes:

- Acute lymphoblastic leukemia/lymphoma (L1 and L2);
- Precursor B acute lymphoblastic leukemia/lymphoma;
- Precursor T acute lymphoblastic leukemia/lymphoma;
- Burkitt's leukemia/lymphoma (L3);
- Biphenotypic acute leukemia.

Features of hematopoiesis in acute leukemia:

- more than 20 % blasts in the bone marrow (the international diagnostic criteria);
- inhibition of erythro- and megakaryocytopoiesis.

Features of peripheral blood picture in acute leukemia:

- anemia;
- thrombocytopenia;
- the WBC count although usually high, leukocytosis varying from 10 to 200×10^9/l;
- atypical blasts is the main mass of the cells;
- hiatus leukaemicus — the absence of maturating forms between blasts and mature cells;
- increased ESR.

In 30–50 % of cases, the number of WBC and blasts reduced, blasts are rare or absent in the formula (leukopenic or aleukemic type of leukemia).

Clinical stages of acute leukemia:

Acute leukemia in the development passes the following clinical stages:

Debut (first attack) — is the time period from the occurrence of the first clinical and hematological disease symptoms to diagnosis and start of treatment to obtain the effect of the therapy.

Alternating remissions and relapses.

Remission — is weakening the manifestations of the pathological process under the influence of cytostatic therapy. Distinguish between complete and incomplete remission.

Complete remission characterized by:

- normalization of clinical parameters, peripheral blood and bone marrow for at least 1 month;
- bone marrow: blast cells in BM ≤ 5 %, and none can have a leukemic phenotype (eg, Auer rods), lymphocytes < 30 %;
- hemogram: blasts — absent, granulocytes ≥ 1.5×10^9/l, Tr ≥ 100×10^9/l, Hb ≥ 100g/L(male), ≥ 90g/L (female and children);
- clinical: disappear of pathological symptoms;
- subjective: no complaints.

State of complete remission for 5 years or more is called recovery.

Incomplete remission characterized by normalization of clinical parameters, peripheral blood and bone marrow, but blast cells in BM ≤ 20 %.

Relapse — is the return of leukemic process after remission (bone marrow, outside bone marrow, combined).

Bone marrow relapse is divided into:

- aleukemic — blasts in BM > 20 %, in peripheral blood — absence;
- leukemic — blasts in BM > 20 % and in peripheral blood.

Outside bone marrow (local) *relapse* is the presence of leukemic infiltrates outside BM (in lymph nodes, spleen, skin, etc.).

Terminal stage of acute leukemia — is the final stage of tumor progression, comes with complete depletion of normal hematopoiesis and resistance to cytostatic therapy. The most often causes of patient's death are infectious and inflammatory complications, bleeding, hemorrhage in internal organs.

Acute myeloblastic leukemia (AML)

The tumor arises from the transformed progenitor cells of myelopoiesis (II class on modern scheme of hematopoiesis).

Substrate tumor — myeloblasts (IV class on modern scheme of hematopoiesis).

Peripheral blood picture:

- anemia;
- thrombocytopenia;
- myeloblasts is the main mass of the cells;
- hiatus leukaemicus.

Bone marrow: numerous myeloblasts with azurophilic granules and 1-2Auer rods.

Cytochemical features of myeloblasts are presented in table 17.

Table 17 — Cytochemical features of myeloblasts in different type of AML by FAB classification

	M1	M2	M3	M4	M5	M6	M7
MPO	+	+	+	+	–/+	–	–
NSE	–	–	–/+	+	++	+	+/–
PAS	–	–	–	–	–/+	+	+

Manifestations:

Bone marrow failure is due to replacement of normal marrow hematopoietic cells by leukemic blast cells. All or any of the three cell lines are decreased, which results in anemia, neutropenia and thrombocytopenia. Anemia causes fatigue, weakness (directly related to the degree of anemia). Neutropenia results in life-threatening infections by bacteria or opportunistic fungi, Pseudomonas and commensals in different organs and systems. Thrombocytopenia presents as bleeding manifestations in the form of petechiae, atraumatic ecchymoses, gum, nasal, urinary tract bleeding.

Leukostasis — stasis of blood flow may develop when the blast count in the peripheral blood is above 50×10^9/l. It is more common in AML than ALL because myeloblast is larger and expresses adhesive proteins. Cerebral leukostasis may cause headache, confusion and visual disturbances.

Disseminated intravascular coagulation syndrome is observed in AML-M3 (promyelocyte leukemia).

Extramedullary infiltration may be in organs like lymphnodes, spleen, gingiva, skin (leukemia cutis) and meninges. Gingival hypertrophy and infiltration of skin (leukemia cutis) may be found in monocytic type of AML (M4).

Splenomegaly and hepatomegaly may be found and is more common in AML.

Acute lymphoblastic leukemia (ALL)

In adults this tumor is rare; in children — is 80 % of all forms of leukemia. The peak incidence occurs between the ages of 4–5 years.

The tumor arises from the transformed progenitor cells of lymphopoiesis (II class on modern scheme of hematopoiesis).

Substrate tumor — lymphoblasts (IV class on modern scheme of hematopoiesis).

Peripheral blood picture:

- anemia,
- thrombocytopenia,
- lymphoblasts is the main mass of the cells,
- granulocytopenia
- absolute lymphopenia (significantly reduced content of differentiated lymphocytes).

Bone marrow: more than 20 % lymphoblasts restriction normal hematopoiesis.

Manifestations:

Symptoms are related to depressed marrow function due to infiltration by blasts. There are anemia, neutropenia and thrombocytopenia. Lymphadenopathy is present in 75 % of patients. Splenomegaly is more common than hepatomegaly. Leukemic meningitis is rare and is more common in ALL (pre-B). In children with ALL metastases most commonly affects the testicles (in boys), and meninges.

CHRONIC LEUKEMIA

Chronic leukemia — is a neoplasm originating from bone marrow, with partial delay ability of hematopoietic cell to the differentiation. In chronic leukemia cells retain the ability to differentiate to the stage of maturating or mature cells.

Substrate for chronic leukemia — maturating (V class) and mature (VI class) cells.

Classifications of chronic leukemia:

Chronic leukemia is conventionally divided into 2 groups:

- myeloproliferative;
- lymphoproliferative.

The chronic myeloproliferative leukemia include:

1. Chronic myeloleukemia.
2. Chronic monocytic (myelomonocytic).
3. Chronic neutrophilic leukemia.
4. Chronic eosinophilic leukemia / hypereosinophilic syndrome.
5. Essential thrombocythemia (ET).
6. Polycythemia vera (PV) — erythremia.
7. Idiopathic myelofibrosis (subleukemic myelosis).

The chronic lymphoproliferative leukemia include:

Chronic B-cell leukemia:

• B-cells prolymphocyte leukemia;

• Paraproteinemic hemoblastoses:

✓ Multiple myeloma;

✓ Waldenstrom's macroglobulinemia;

✓ Heavy chain disease;

• Hairy cell leukemia.

Chronic T-cell / NK-cell leukemias:

• T-cells prolymphocyte leukemia;

• T-cells leukemia of large granular lymphocyte;

• Aggressive NK-cell.

Features of hematopoiesis in chronic leukemia:

• partially delayed cell maturation;

• increases content of myeloid cells, particularly myelocytes metamyelocytes neutrophils in CML;

• occurs infiltration by mature small lymphocytes in CLL.

Features of peripheral blood picture in chronic leukemia:

• anemia and thrombocytopenia develop as the disease progresses;

• WBC count varies significantly depending on the stage (from moderate leukocytosis to a significant increase in the number of leukocytes);

• as a result of chronic leukemia progression in the bone marrow and in the blood can be increased the number of blast cells (up to blastic crisis);

• CML is characterized by basophilic-eosinophilic association (simultaneous increase in the absolute number of eosinophils and basophils in the peripheral blood).

Clinical stages of chronic leukemia:

Chronic (expand) — is characterized by a long-compensated current.

Acceleration — is characterized by active proliferation of leukemic cells.

Terminal — occurs blast transformation, is manifested by blast crisis.

Blast crisis — is a sharp increase in the number of blast cells in bone marrow and peripheral blood (more than 20 %), progression of anemia, thrombocytopenia and formation of out bone marrow leukemic infiltrates. Blast crisis is the final phase of chronic leukemia and its clinical and hematological picture corresponds to the picture of acute leukemia.

Hematological criteria of blast crisis (WHO, 2008):

• in the bone marrow and peripheral blood more than 20% blast cells;

• extramedullary blastic proliferation;

• blast infiltration of the bone marrow histologically detectable.

Chronic myeloid leukemia (CML)

Tumor arises from hematopoietic stem cells (I-II classes on modern scheme of hematopoiesis). Inhibition of differentiation occurs at the level of maturating granulocytes.

Substrate tumor — mature neutrophils, metamyelocytes, myelocytes, promyelocytes, myeloblasts (a little) (V–VI classes on modern scheme of hematopoiesis).

Peripheral blood picture in chronic phase:

- hemoglobin, RBC count is normal, later — normochromic anemia;
- platelets are normal, possible of their increase or decrease;
- neutrophilic leukocytosis with a left shift to promyelocytes, single blast;
- basophilic-eosinophilic association.

With tumor progression to the acceleration and terminal stage increases anemia, thrombocytopenia, increases the number of blasts. In phase of blast crisis there is marked neutropenia, thrombocytopenia and pronounced increase of blasts more than 20 %.

Bone marrow:

- bone marrow is hypercellular due to granulocyte increased leuko/ erythroblastic ratio;
- in progress is the oppression of erythro- and megakaryocytopoiesis;
- progressively increasing the number of blast cells.

Manifestations*:*

Most of patients are diagnosed in the chronic phase. Common symptoms include fatigue, weight loss, low-grade fever, bone tenderness, abdominal fullness, abdominal pain (splenic infarcts), loss of appetite, night sweats, and splenomegaly. As the disease progresses, the features worsen. Appearance of blasts and promyelocytes in the peripheral blood increase splenomegaly, bone pain, thrombocytopenia (bleeding, petechiae, ecchymosis and bruising) and worsen anemia. Many patients are asymptomatic.

Erythremia (Vakeza disease, Polycythemia Vera)

Polycythemia Vera is a myeloproliferative disorder marked by erythrocytosis (increased red cell mass). The tumor arises in the transformation of the stem cell or progenitor cells of myelopoiesis.

Substrate tumor — may be the cells of 3 or 4 hemopoietic lineage: granulocytic, monocytic, erythroid, megakaryocytic.

Peripheral blood picture:

- erythrocytosis is the most prominent clinical manifestation (with a reduction of erythropoietin levels in blood and urine);
- hemoglobin increased to 180–220 g/l;
- reticulocytosis;
- can be thrombocytosis with giant forms;
- neutrophilic leukocytosis with a left shift to myelocytes;
- sharply lower ESR, hematocrit and blood viscosity increased.

Bone marrow: hypercellular with hyperplasia of all three bone marrow elements. In greatest degree damaged erythroid lineage. Decrease leuko/erythroblastic ratio due to erythroid hyperplasia.

***Manifestations**:*

Most commonly occurs in older adults (average age = 60 years). Many of symptoms are related to sluggish blood flow caused by increased blood viscosity (increased hematocrit). Headaches, pain in the joints, spine and fingertips are associated with microvascular occlusion. It is also noted hypertension, splenomegaly (80 % of patients). Thrombotic complications are common and a major factor in morbidity.

Chronic lymphoid leukemia (CLL)

In 95 % of cases CLL malignant transformation is undergoing a «naive» B-lymphocyte; in remaining 5 % of cases — a common lymphoid progenitors. The chronic malignant lymphoproliferative disorders originate in the bone marrow and slowly progress to involve the peripheral blood, lymph nodes, spleen, and liver.

Substrate tumor — proliferating mature and maturating lymphocytes, may be of B, T, or NK origin.

Peripheral blood picture:

- anemia, thrombocytopenia, (due to the displacement of germs from the bone marrow, and due to the formation of antibodies to them by leukemia cells);
- more often observed leukocytosis;
- absolute lymphocytosis (> $5{,}0\times10^9$/l, but usually > $15{,}0\times10^9$/l and sometimes > $100{,}0\times10^9$/l);
- may be prolymphocytes (from single up to 5–10 %) and lymphoblasts;
- Botkin-Gumprecht shadow (destroyed in the preparation of a smear leukemic lymphocytes) — a characteristic hematological symptom of CLL.

Bone marrow:

- often diffusely replaced by small lymphocytes. Lymphocyte amount exceeds 30 %, in severe cases it may be higher;
- granulocyte, erythroid, monocyte and megakaryocytic germs are narrowed;
- interstitial or nodular lymphocytes infiltration.

***Manifestations**:*

CLL is a disease of adults; most patients are > 60 years of age.

Leukemic lymphocytes have decreased ability to reaction of blast transformation (transformation of B lymphocytes into plasma cells) and appear ability to synthesize of Ig with distorted features. As a result CLL is often accompanied by microbial complications and autoimmune hemolytic anemia.

Lymphadenopathy and splenomegaly are common especially late in the disease because small lymphocytes accumulate in the marrow, spleen, lymph nodes and liver.

The clinical course is highly variable with survival ranging from 1–20 years.

LYMPHOMAS

The malignant lymphomas constitute a heterogeneous group of neoplasms arising from the immune system and primarily involving lymphoid cells.

Classification of lymphomas

WHO-classification of tumors of lymphoid tissue (2008) allocates more than 30 varieties of mature B-cell neoplasms, and over 20 varieties of mature T / NK-cell neoplasms.

Lymphomas are classified based on the cell type and the architectural (growth) pattern: the Hodgkin and the Non-Hodgkin lymphomas.

Hodgkin Lymphoma (HL)

Classification of Hodgkin lymphoma WHO (2008):

- Nodular lymphocyte predominant Hodgkin lymphoma.
- Classical Hodgkin lymphoma.
- Nodular sclerosis classical Hodgkin lymphoma.
- Lymphocyte-rich classical Hodgkin lymphoma.
- Mixed cellularity classical Hodgkin lymphoma.
- Lymphocyte-depleted classical Hodgkin lymphoma.

Manifestations

Hodgkin lymphoma has a unique bimodal distribution, with the first peak being between the ages of 15 and 34 years and a second peak among individuals over 50 years.

HL is a lymph node-based malignancy and commonly presents as an asymptomatic lymphadenopathy that may progress to predictable clinical sites. More than 80 % of patients with HL present with lymphadenopathy above the diaphragm, often involving the anterior mediastinum; in about 30 % may be involved the spleen. Less than 10 % to 20 % of patients present with lymphadenopathy limited to regions below the diaphragm.

Table 18 — The Cotswold staging classification for Hodgkin lymphoma

Stage	Description
I	Involvement of a single lymph node region or lymphoid structure (eg, spleen, thymus, Waldeyer's ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (ie, the mediastinum is a single site, hilar lymph nodes are lateralized).
III	Involvement of lymph node regions or structures on both sides of the diaphragm. With or without involvement of splenic, hilar, celiac, or portal nodes. With involvement of para-aortic, iliac, or mesenteric nodes
IV	Involvement of extranodal site(s) beyond that designated

The commonly involved peripheral lymph nodes are located in the cervical, supraclavicular, and axillary areas; para-aortic pelvic and inguinal areas are involved less frequently. Lymph node is painless enlargement, usually firm or rubbery, often multiple and fixed in place. Disseminated lymphadenopathy is rare.

Systemic symptoms include fever, persistent fatigue, night-sweats (drenching sweats of the whole body), and weight loss. As lymphoma progresses, spread may occur to spleen, liver, bone marrow, and other organs.

HL is characterized by the presence of Reed-Sternberg cells (large size and classic binucleated structure with large eosinophilic nucleoli, with CD30 and CD15 markers).

Non-Hodgkin Lymphoma (NHL)

Classification of NHL

In the structure of Non-Hodgkin lymphomas is dominated by the B-cell neoplasms. The most common options are: diffuse large B-cell lymphoma (30 % of all cases of NHL), follicular lymphoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), CLL/small lymphocytic lymphoma, mantle cell lymphoma, Burkitt's lymphoma, nodal marginal zone lymphoma, lymphoplasmacytic lymphoma (Waldenstrom macroglobulinemia). Among the mature T / NK-cell neoplasms the most common variant is peripheral T-cell lymphoma (8 % of the non-Hodgkin lymphoma).

Etiology

Unclear. The most commonly associated chromosomal abnormality in NHL is the t(14;18)(q32;q21) translocation. It is found in 85 % to 90 % of follicular lymphomas and 25 % to 30 % of higher-grade B-cell NHLs. Chromosomal translocations involving 8q24 (lead to c-myc deregulation) is seen in nearly all cases of Burkitt lymphoma.

Risk Factors:

- the incidence of NHL progressively increases with age (peak approximately 60 years);
- environmental factors (pesticides and herbicides (2,4-D-organophosphates, chlorophenols), solvents and organic chemicals (benzene, carbon tetrachloride, trichloroethylene));
- viruses (EBV, HTLV-1, Kaposi sarcoma-associated herpesvirus (human herpesvirus 8) and hepatitis C virus;
- bacterial infections (Borrelia burgdorferi (in Lyme disease), has been detected in about 35 % of patients with peripheral cutaneous B-cell lymphoma; Campylobacter jejuni and α-heavy chain disease are related);
- Congenital immunodeficiency (ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable hypogammaglobulinemia, X-linked lymphoproliferative syndrome and severe combined immunodeficiency) and acquired immunodeficiency (HIV infection).

Manifestations

Fever, weight loss, and night sweats, referred to as systemic B symptoms, as well as fatigue and weakness. The NHLs are a heterogeneous group of neoplasms that usually arise or present in lymphoid tissues, such as lymph nodes, spleen, and bone marrow, but they may arise in almost any tissue. Splenomegaly is seen in about 40 % of patients. The most frequent sites for extranodal lymphomas are the stomach, skin, oral cavity and pharynx, small intestine, and central nervous system. Bone marrow is frequently involved, sometimes in association with cytopenias.

Peripheral blood: lymphocytosis with circulating malignant cells.

Bone marrow and peripheral blood involvement may be present, and the distinction between leukemia and lymphoma is difficult to make in some cases.

PARAPROTEINEMIC HEMOBLASTOSIS

Paraproteinemic hemoblastosis — group of diseases characterized by monoclonal proliferation of B-lymphoid cell secreting monoclonal Ig (or their fragments) are determined in serum and / or urine (paraproteins).

Paraproteins correspond to various variants of normal immunoglobulins (IgG and often IgM), but differ in uniformity of light and heavy chains or are structurally abnormal immunoglobulin molecules (fragments of heavy or light chains).

The main feature of these leukemias is to preserve the ability of B cells differentiate to the stage of immunoglobulin-secreted cells.

Paraproteinemic hemoblastoses include:

- Multiple myeloma;
- Waldenstrom's macroglobulinemia;
- Heavy chain disease (HCD).

Multiple myeloma (MM)

In multiple myeloma progenitor of B-lymphocytes undergo malignant transformation, but retains the ability to maturate plasma cells. Plasma leukemic cells actively proliferate in the bone marrow and secrete myeloma paraprotein (Ig) — M-protein that enters the blood or urine.

Peripheral blood picture:

- anemia;
- often the first sign is a significant increase in the ESR to 50–90 mm/h (not for all biochemical variants of multiple myeloma);
- WBC are normal, at progress of disease may develop leukopenia;
- may appear single plasma cells (large numbers in the terminal stage).

Bone marrow: infiltration by plasma cells and oppression of normal hematopoiesis. The content of plasma cells in the bone marrow more than 10 % — is one of the basic disease diagnostic criteria.

Manifestations*:*

MM occurs in older age groups, affects men more than women. Plasma cells are able to synthesize factors activating osteoclasts. Due to activation of the

latter is destroyed bone tissue, may occur spontaneous fractures, hypercalcemia and associated lesions of the nervous, cardio-vascular systems, kidneys, gastrointestinal tract.

Reduced amount of normal plasma cells leads to disruption of formation normal Ig and syndrome of recurrent infections.

Excessive formation of immunoglobulin light chains leads to kidney damage (nephropathy myeloma). It is a 2nd place of complications leading death cause in multiple myeloma after infectious.

Bence Jones protein is a peculiar protein made by some patients with MM as a result of an excess of kappa and lambda light chains. These light chains are small and can be filtered by the kidneys, detected in the urine (proteinuria Bence-Jones).

On proteins electrophoregram in 80 % of patients is determined a characteristic monoclonal peak (M-gradient). But in Bence Jones myeloma and non-secretory myeloma the serum M-gradiet can not be detected.

Waldenstrom macroglobulinemia

It is characterized by production of macromolecular monoclonal paraproteins IgM by neoplastic B cells.

Peripheral blood picture:

- anemia;
- thrombocytopenia may develop at disease progress;
- possible neutropenia;
- monocytosis;
- ESR always significantly increased.

In blood serum — hyperproteinemia, electrophoregram — M gradient due to IgM.

Urine analysis: Bence-Jones protein occurs in approximately 80 % of cases, but its amount is significantly less than in multiple myeloma.

Bone marrow: contains variable numbers of pleomorphic lymphoid cells.

Manifestations*:*

Due to the accumulation of high molecular proteins are characterized by an increase in blood viscosity, disturbance of microcirculation, predisposition to thrombosis, hemorrhagic syndrome. Usually found lymphadenopathy and hepatosplenomegaly.

Heavy chain diseases

These are B-cell lymphatic tumors with various clinical and morphological picture and secretion of heavy chains fragments (α-,γ-, μ-, δ- chains) of different Ig classes:

- α-chain disease (Seligmann's disease);
- γ-chain disease (Franklin disease);
- μ-chains disease;
- δ-chains disease.

***Manifestations**:*

Alpha heavy chain disease typically affects the gastrointestinal system; rare cases of respiratory and lymphomatous forms have been reported. The abdominal form of alpha heavy chain disease presents as a malabsorption syndrome with weight loss, diarrhea, and abdominal discomfort due to diffuse infiltration of the small intestine mucosa and mesenteric lymph nodes by plasma cells, macrophages, and mast cells. Generalized lymphadenopathy and hepatosplenomegaly are hallmarks of the lymphomatous form. Pulmonary form is characterized by bronchopulmonary lesions and mediastinal lymphadenopathy. Proteinuria is absent.

Gamma heavy chain disease associated with rheumatoid arthritis (the most common), systemic lupus erythematosus, vasculitis, and myasthenia gravis, as well as autoimmune cytopenias, including idiopathic thrombocytopenic purpura. Manifestations of autoimmune disease often precede gamma heavy chain disease by many years.

The symptoms and signs of mu heavy chain disease are related to the associated lymphoma. Splenomegaly is almost universally present, with hepatomegaly in three-quarters of patients.

Principles of leukemia diagnosis

Identify the type of leukemia suppose a comprehensive approach and carried out by means:

- morphological;
- cytochemical;
- immunophenotyping;
- cytogenetic and molecular genetic methods.

Study the picture of peripheral blood and bone marrow (to confirm the diagnosis in suspected leukemia). Diagnosis of leukemia may also include biochemical analysis of blood serum or urine, lumbar puncture, ultrasound, x-ray, computed tomography scan and magnetic resonance imaging.

Principles of leukemia therapy

Specific chemotherapy — aims to achieve and maintain remission of the disease; consists of several stages, different for lymphoblastic and myeloid leukemia and perform according to standard schemes.

Accompanying therapy — aimed at fighting infections, reduction of toxicity in tumor lysis syndrome, reduction of toxic side effects of chemotherapy drugs.

Replacement therapy — is needed in a threatening thrombocytopenia, severe anemia, blood clotting disorders. It includes transplantation of hematopoietic stem cells, or bone marrow.

CHAPTER 6
PATHOLOGY OF HEMOSTASIS SYSTEM

Hemostasis system — is a combination of biological and biochemical mechanisms that maintain liquid circulating blood, maintaining the integrity of blood vessels and arresting bleeding when they are damaged.

Microcirculation in organs and tissues and level of their blood supply to a large extent depends on the functioning of the hemostatic system. The pathology of this system is manifested by bleeding, development of thrombosis, infarcts and ischemia of organs. Hemostasis is carried out by three interacting with each other morphological and functional components: the walls of blood vessels, blood cells (primarily platelets) and plasma systems — coagulation, anticoagulation, fibrinolytic (plasmin) and kallikrein-kinin.

Blood vessels and platelets the first react to injury (primary hemostasis), followed by blood coagulation occurs with the participation of plasma factors (secondary), although both of these mechanisms are mutually potentiate each other and function conjugately.

THROMBOCYTOPOIESIS

First morphologically recognizable cell of this series is megakaryoblasts. Further development of the cells is carried out by endomitotic way, occurs repeatedly doubling the number of chromosomes without cell division. Formed promegakaryocyte, then megakaryocyte — representing the polyploid cells with the number of nuclei, reaching 128 (the main part of megakaryocytes contain 8–16 nuclei). The process of converting megakaryoblasts in megakaryocytes lasts about 25 hours. The life cycle of megakaryocytes is about 10 days. Platelet production is carried out by the bud off cytoplasm from the megakaryocytes.

COAGULATIVE AND ANTICOAGULATIVE SYSTEMS

Platelet clotting factors

Factor 1 — platelet accelerator globulin identical factor V;

Factor 2 — accelerator thrombin, fibrin plastic factor (accelerates the conversion of fibrinogen);

Factor 3 — platelet thromboplastin, partial thromboplastin;

Factor 4 — anti-heparin factor;

Factor 5 — clotting factors (immunologically identical to fibrinogen);

Factor 6 — trombostenin;

Factor 7 — platelet co-thromboplastin;

Factor 8 — anti fibrinolysin;

Factor 9 — fibrin-stabilizing factor, by the action corresponds to factor XIII;

Factor 10 — 5-hydroxytryptamine, serotonin;

Factor 11 — adenosine diphosphate (ADP).

Table 19 — Plasma coagulation factors

Number, trivial name	Function	Place of formation	Activation factors and mechanism of action
I fibrinogen	Structural protein	Hepatocytes	Converted by thrombin to fibrin (Ia — basic substance of thrombus). Participates in platelet aggregation. Promotes tissue repair
II prothrombin	Zymogen of serine protease of thrombin	Hepatocytes	Under the influence of active prothrombinase converted into thrombin (IIa). Activates fibrinogen to form fibrin
III Tissue thromboplastin or apoprotein III	Transmembrane protein	Endothelial cells, macrophages Released at injury of the vessel wall, tissues	Cofactor of factor VII, triggers the extrinsic pathway of blood coagulation
IV calcium ions — Ca^{2+}		Platelet granules (dense bodies), absorbed from the intestine	Participates in the formation of complexes of plasma factors and lipids. Is part of the active prothrombinase. Promotes platelet aggregation. Bind heparin. Participates in the formation of the primary hemostatic plug and thrombus retraction. Inhibits fibrinolysis
V Proaccelerin, labile factor, accelerator (Ac-) globulin	Protein cofactor	Hepatocytes, megakaryocyte, platelet	Activated by factor IIa. Is part of the active prothrombinase. Creates optimal conditions for the interaction of factor Xa and II
VII Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin or stable factor	Zymogen of serine protease	Hepatocytes (in the presence of vitamin K)	Activated by factor III. Activates factors IX, X (involved in the formation of prothrombinase in external path)
VIII: Antihemophiliac factor A, antihemophilic globulin (AHG)	Protein cofactor	Hepatocytes	Activated by thrombin. Creates optimal conditions for the interaction of factors IXa and X
von Willebrand factor	Structural protein	Endotheliocyte, megakaryocyte	Stabilizes factor VIII. Promotes platelet adhesion
IX Christmas Factor, antihemophilic factor B, plasma thromboplastin component (PTC)	Zymogen of serine protease	Hepatocytes (in the presence of vitamin K)	Activated by factor IXa, VIIa. Activates factor X
X Stuart-Prower Factor	Zymogen of serine protease	Hepatocytes (in the presence of vitamin K)	Activated by factor VIIa and VIIIa. Is part of the active prothrombinase. Passes prothrombin to thrombin (IIa)

Number, trivial name	Function	Place of formation	Activation factors and mechanism of action
XI Plasma thromboplastin antecedent (PTA)	Zymogen of serine protease	Hepatocytes	Activated by factor XIIa. Activates factor IX
XII Hageman Factor or contact factor	Zymogen of serine protease	Hepatocytes	Activated by kallikrein and HMWK. Starts the intrinsic pathway of blood coagulation. Activates the PPK, system of fibrinolysis
XIII Protransglutaminase, fibrin stabilizing factor (FSF), fibrinoligase	Zymogen of transglutaminase	Hepatocytes, megakaryocyte	Activated by thrombin and Ca^{2+}. Stabilizes fibrin. Promotes tissue repair
Plasma prekallikrein (PPK) or Fletcher factor	Zymogen of plasma kallikrein	Hepatocytes	Activated by HMWK, factor XIIa. Activates factor VII, XII, HMWK, plasminogen
Fitzgerald factor or high molecular weight kininogen (HMWK)	Glycoprotein	Hepatocytes	Activates factor XI, XII plasminogen, PPK

The mechanism of vascular-platelet hemostasis

Activation of vascular-platelet (primary) hemostasis makes a complete stop of bleeding from capillaries and venules and temporary stop of bleeding from veins, arterioles and arteries by forming the primary hemostatic plug from which upon activation of the secondary (coagulation) hemostasis formed thrombus.

Key mechanisms of thrombosis are: damage of vascular endothelium; local vasoconstriction; adhesion of platelets to the site of naked subendothelium; platelet aggregation; activation of blood clotting while reducing its lytic properties.

1. Damage of endothelium and the primary vasospasm

Microvessels respond to damage by short-term spasm, causing them bleeding does not occur in the first 20–30 seconds. Reflex spasm of blood vessels by contraction of smooth muscle cells of the vascular wall and supported by vasospastic agents secreted by the endothelium and platelets (serotonin, TxA2, norepinephrine, and others).

Endothelial damage is accompanied by a decrease in of the vascular wall and thromboresistance naked subendothelium that contains collagen and expresses the adhesion proteins — von Willebrand factor, fibronectin, thrombospondin.

2. The adhesion of platelets to the site of naked subendothelium

Carried out in the first few seconds after endothelial damage due to:

- reducing the amount of surface negative charge of the vascular wall in violation of its integrity;
- platelets receptor to collagen.

The stabilization of the resulting compound is carried adhesion proteins — von Willebrand factor, fibronectin and thrombospondin, forming «bridges» between their complementary platelet glycoprotein and collagen.

3. Activation of platelets and secondary vasospasm

The activation of platelets is caused by thrombin that formed from prothrombin under the influence of tissue thromboplastin, PAF, ADP (released together with thromboplastin at the vascular wall damage), Ca^{2+}, adrenaline.

The process of platelets activation is associated with the chemical modification of membranes and induction of enzyme glycosyltransferase, phospholipase A2 in them. Glycosyltransferase interacts with specific receptor on the molecule of collagen and thereby provides «landing» of platelets on the subendothelium.

Phospholipase A2 starts the hydrolysis of phosphatidylethanolamine, that lead to the release of arachidonic acid. From arachidonic acid by the action of COG formed prostaglandins PgG2, PgH2, that transforming to the TxA2 (a potent inducer of platelet aggregation and vasoconstrictor) under the influence of an enzyme thromboxane synthetase. Prostaglandins contribute to the accumulation of cAMP in platelets, regulate protein phosphorylation and activation of calmodulin, that transporting Ca^{2+} from the dense tubular system of platelets into the cytoplasm. As a result, there is an activation of contractile protein of actomyosin complex, which is accompanied by a contraction of microfilaments of platelets with the pseudopodia formation. This further enhances platelet adhesion to the damaged endothelium.

At the same time, by the Ca^{2+}-induced contraction of microtubules occurs emptying of granules at two phases: the first phase — release the contents from dense granules, the second — α-granules. TxA2 and dismissed from the dense granules of platelets vasoactive substances cause secondary vasospasm.

4. Platelets aggregation

During degranulation of platelets are released TxA2, ADP, serotonin, β-thromboglobulin, platelet factor 4, fibrinogen and others components of dense granules and α-granules. They cause sticking of platelets together and with collagen. In addition, the appearance of the paf in the bloodstream (at endothelial destruction) and components of platelet granules leads to the activation of intact platelet, their aggregation with platelets that adherent on the endothelium.

Platelets aggregation does not develop in the absence of extracellular Ca^{2+}, fibrinogen.

5. The formation of hemostatic plug

As a result of platelet aggregation formed primary (temporary) hemostatic plug that obturate vascular defects (does not contain fibrin). Subsequently, on the surface of platelets aggregate adsorbed plasma coagulation factors and starts the «internal cascade» of coagulation that ends by the precipitation of stabilized fibrin fibers and the formation of platelet plug on the basis of a blood clot (thrombus).

At contraction of platelets thrombosthenin the thrombus compacted (clot retraction).

«External cascade» of blood clotting involves the release of tissue thromboplastin. Additionally, platelets can independently (in the absence of contact factors) start of blood clotting by the interaction of the factor Va (exposed on their surface) with factor Xa of plasma, catalyzes the conversion of prothrombin to thrombin.

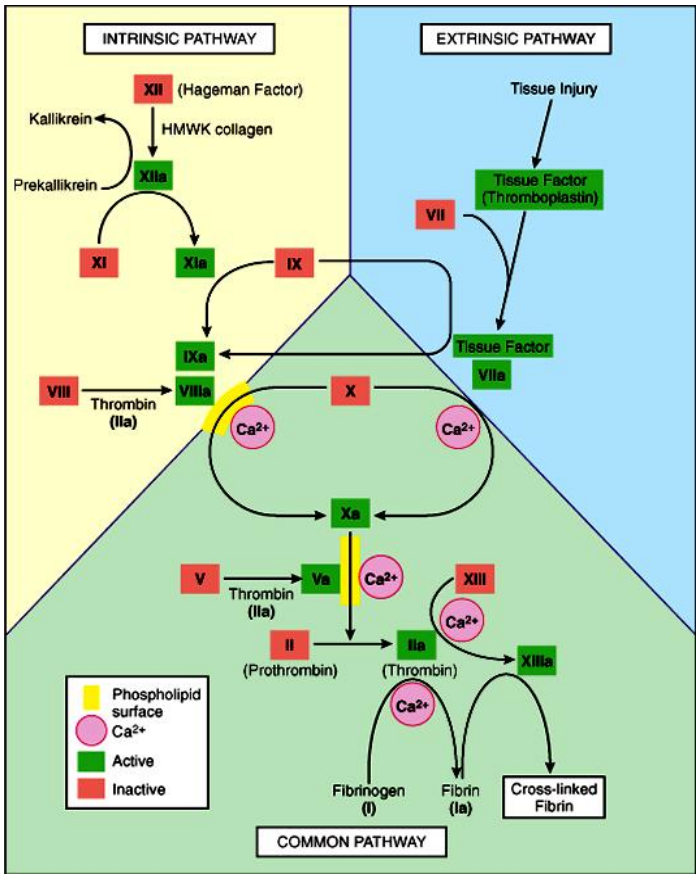


Figure 6 — Sheme of hemostasis (V. Kumar et al., 2010)

The mechanism of coagulation hemostasis

There are three stages of the blood clotting process. The first stage ends with the formation of active prothrombinase complex on membrane phospholipids, which includes factors X, V, and Ca^{2+} by internal and external mechanisms. The second stage is characterized by the formation of thrombin (the active form of factor II). In the third stage (final phase of blood clotting) there is a formation and stabilization of fibrin clot.

Normal hemostasis is attained by cooperation and interaction of primary (vascular platelet) and secondary (coagulation) mechanisms of hemostasis impairments. Causes of hemostasis impairments are hereditary and acquired.

Regulatory Mechanisms

Several inhibitory mechanisms prevent activated coagulation reactions from amplifying uncontrollably, causing extensive local thrombosis or disseminated intravascular coagulation. These mechanisms include:

- inactivation of coagulation factors;
- fibrinolysis;
- hepatic clearance of activated clotting factors.

Inactivation of coagulation factors:

The liquid state of the blood is maintained due to its movement (lowering the concentration of the reactants), the adsorption of the coagulation factors by the endothelium, and independently synthesized and constantly founded in blood the primary anticoagulants (table 20).

Table 20 — Primary anticoagulants and mechanisms of their action

Name	Mechanism of Action
Inhibitor of external coagulation pathway	Inhibits «PF + VIIa + Xa + Ca^{2+}» complex
α_2-macroglobulin	Synthesized by hepatocytes inhibitor of «PF + viia» complex, thrombin, plasmin, kallikrein
α_1-antitrypsin	Synthesized by hepatocytes inhibitor of trypsin, thrombin, plasmin, kallikrein, has to 90–92 % of the total anti-protease activity of plasma
Thrombomodulin	Receptor protein of endothelial cells, binds and inactivates thrombin; complexed with thrombin activates protein C and S
Antithrombin III	Neutralizes thrombin, factor Xa and ixa; plasma heparin cofactor
Heparin cofactor II	Forms a complex with heparin; active in the plasma devoid of antithrombin III
Heparin	Being a component of the vascular wall, activates antithrombin III, in a complex with antithrombin III inhibits thrombin stimulates release of inhibitor of external coagulation pathway by endothelial cells
Protein C	Synthesized by hepatocytes vitamin K-dependent inhibitor of factors Va and viiia (when activated by thrombin and complex «thrombomodulin + thrombin» in interaction with protein S) stimulates fibrinolysis (endogenous plasminogen activator)
Protein S	Synthesized by hepatocytes vitamin K-dependent Protein C cofactor involved in the proteolytic degradation of factors Va and viiia
Inhibitors of fibrin monomers polymerization	Inhibit the fibrin polymerization

Many of clotting factors and their fragments, formed during coagulation, act as secondary anticoagulants. In particular, the anticoagulant effects have fibrin and degradation (by plasmin) products of fibrinogen, inhibiting the final phase of blood coagulation. In pathological conditions in the blood may occurs immune inhibitors of blood coagulation factor in high titer — antibodies to factor VIII, IX etc. and to the membrane phospholipids, on which clotting factors is interact and activates.

Fibrinolysis

Fibrin deposition and lysis must be balanced to maintain temporarily and subsequently remove the thromb during repair of an injured vessel wall. The blood fibrinolytic system comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin. Plasmin degrades fibrin into soluble fibrin degradation products, by two physiological plasminogen activators (tissue and urokinase type). Plasminogen activators cleave plasminogen (from

plasma) into plasmin. Plasmin proteolyze fibrin into soluble fibrin degradation products. They are swept away in the circulation. Fibrinolysis is controlled by inhibitors of plasminogen activator and plasmin inhibitors (eg, α2-antiplasmin).

Classification of hemostasis disorders:

By pathogenesis:

- change in quantity and properties of thrombocytes;
- coagulopathias;
- vasopathia.

By the character of impairments:

- hypocoagulative (hemorrhage syndrome);
- hypercoagulative (thrombotic syndrome);
- complex impairments (thrombotic-hemorrhage conditions).

Reasons for hypocoagulation are united in four groups:

- thrombocytopenia;
- thrombocytopathy;
- vassopathy (angiopathy);
- coagulopathy.

CHANGE IN QUANTITY AND PROPERTIES OF THROMBOCYTES

In nome platelet count is $150–450\times10^9$/l. Violations of platelet hemostasis include:

- thrombocytopenia;
- thrombocytosis;
- thrombocytopathy.

Thrombocytopenia

Thrombocytopenia — is a decrease in the number of thrombocytes below 150×10^9/l. Hemorrhagic syndrome depends on degree of thrombocyte number decrease. Clinical signs of thrombocytopenia are manifested in the decrease of thrombocytes number below 50×10^9/l and include: gum bleeding, menorrhagias, the increased ability of incutaneous bleedings, and appearance of pethechias of different localization. There are gastric and nasal bleedings in severe cases.

Classification of thrombocytopenia:

- independent disease;
- symptoms of various diseases;
- hereditary;
- acquired.

Mechanisms of heredity thrombocytopenia:

- insufficient number of megakaryocytes in BM (Fanconi syndrome, cyclic amegakaryocytic thrombocytopenia);

- inefficient thrombocytopoiesis;
- defect of synthesis of thrombopoietin;
- megakaryocyte dystrophy syndrome («gray» platelet anomaly Mey-Hegglina).

Mechanisms of acquired thrombocytopenia:

1. Decreased intensity of production in BM:

- hypo-or aplasia of hemopoiesis;
- IR, chemical substances;
- substitution BM by tumor tissue;
- inefficient thrombocytopoiesis.

2. Increased of thrombocytes destruction out of BM:

- immune:

✓ transimmune (through placenta in Verlgof disease);

✓ heteroimmune (Ab to hapten fixed on thrombocytes);

✓ isoimmune (incompatibility by Ag of mother and fetus thrombocytes or donor and recipient during hemotransfusions);

✓ autoimmune (idiopathic, with lymphoproliferative disease);

- non-immune:

✓ syndrome of hypersplenism;

✓ mechanical trauma of thrombocytes (catheters, prosthetic heart valves).

3. Increased consumption of thrombocytes:

- consumption coagulopathy (HUS, ITP, hemorrhagic vasculitis);
- thrombophilia.

4. Dilution.

5. Redistribution.

Decreased production of platelets is caused by the impairment of thrombocyte formation. In patients with vitamin B_{12} or folic acid deficiency, there is accelerated destruction of megakaryocytes, in the bone marrow (ineffective megakaryopoiesis) because DNA synthesis is impaired. It is observed in case of bone marrow inhibition by cytotoxic drugs, radiation, thiazides, estrogenes, alchohol, in bone marrow infiltration in leukemia, disseminated cancer.

Consumption develops in case of the increased platelet utilization above the bone marrow compensatory possibilitiy. This is a cause of thrombocytopenia developing by immunologic and non-immunologic mechanisms. Immunologic destruction: thrombocytopenic purpura in children and adults, neonatal alloimmune purpura. Nonimmunologic destructioh: DIC, sepsis, endothelial injury by viruses.

Dilution develops in massive fluids transfusions after severe blood loss.

Redistribution is observed in patients who have a marked splenomegaly and an increase in platelet pool of spleen.

Manifestations of thrombocytopenia

Bone marrow:

- Hyperplasia with an increase in the number of megakaryocytes and megakaryoblasts (with increased destruction or generalized «consumption» of platelets).

• Hypoplasia (in patients with leukemia, radiation sickness, tumor metastasis in bone marrow).

• Decrease in glycogen content and activity of LDH, G-6-PD in megakaryoblasts and megakaryocytes, which reduces the life span of platelets.

Peripheral blood:

• Reduction of the platelets number and an increase in their size, generally normal RBC, Hb, WBC count.

• In patients with severe hemorrhagic syndrome may develop anemia.

Thrombocytosis

Thrombocytosis — characterized by an increase in the number of platelets per unit volume of blood above 450×10^9/l.

Thrombocytosis divided into:

• primary (tumor, essential);
• secondary (reactive);
• absolute;
• relative;
• symptomatic,
• idiopathic.

Primary thrombocytosis — arise from malignant transformation of megakaryoblasts or hematopoietic stem cells with the subsequent intensification thrombocytopoiesis (CML, AML, erythremia, essential thrombocythemia).

Secondary thrombocytosis — result from increasing the concentration or activity the stimulants of thrombocytopoiesis: thrombospondin, thrombopoietin, PAF, IL-3, IL-6, IL-11; genesis is not associated with damage to hematopoietic cells. Develop after massive bleeding, after splenectomy in chronic inflammation of various etiologies (rheumatoid arthritis, osteomyelitis, tuberculosis), hypercortisolism and stress.

Absolute — are characterized by an increase in the number of platelets in the blood as a result of their increased formation.

Relative — does not accompanied by an increase in the number of blood platelets:

• *Redistributive* (increase in the number of platelets in the areas of microvessels with damaged walls, due to the ejection of blood from the depot and exit from bone marrow).

• *Hemoconcentration* (due to dehydration).

Symptomatic — a symptom of myeloproliferative diseases (chronic myeloid leukemia, erythremia, myelofibrosis, acute leukemia megacaryoblastic et al.).

Idiopathic — an independent nosological form (essential thrombocythemia).

Manifestations of thrombocytosis:

Thrombotic syndrome — excess blood clots with significant thrombocytosis.

Hemorrhagic syndrome — occurs in leukemia along with thrombosis/ due to platelets tumor clones may be functionally defective. Also hemorrhagic syndrome may develop compensatory through increased anticoagulant activity and fibrinolysis.

Thrombocytopathia

Thrombocytopathy — pathological conditions characterized by platelet dysfunction. Unlike thrombocytopenia, thrombocytopathia is characterized by stable functional, biochemical and morphologic changes in platelets and normal platelet count.

Classification of thrombocytopathia

- Hereditary (primary):
- ✓ Glanzmann thrombasthenia;
- ✓ Von Willebrand disease;
- ✓ Bernard-Soulier disease;
- Acquired (secondary) or symptomatic:
- ✓ In diseases and syndromes (leukemia, liver and kidney diseases, lack of vitamins B_{12}, C);
- ✓ iatrogenic (under the influence of medicines).

Pathogenesis of thrombocytopathy includes violation:

- the synthesis and accumulation of BAS in platelets granules;
- degranulation and release of platelet factor in the blood plasma;
- the structure and properties of the platelet membrane.

Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare autosomal recessive bleeding syndrome affecting the megakaryocyte lineage and characterized by lack of platelet aggregation.

Etiology: absence or defect in the membrane receptor for fibrinogen and glyco-proteins IIb–IIIa.

Pathogenesis: reduction in the intensity of fibrinogen binding to the membrane of thrombocytes, violation of thrombocytes aggregation.

Manifestations:

- petechial ecchymoses bleeding type;
- a tendency to bleeding from the mucous membranes (nasal, uterine, hemorrhage in sclera and retina);
- prolonged bleeding after tooth extraction, surgical manipulations.

COAGULOPATHY

Coagulopathy are hereditary (hemophilia A, B, C, Von Willebrand disease et al.) and acquired (DIC syndrome). Among the hereditary bleeding disorders dominate (about 97 %) haemophilia A and B.

Hereditary coagulopathy

Hemophilia A

Etiology: a deficiency of factor VIII (antihemophilic globulin). Inherited X-linked recessive affected male persons (10 per 100 thousand men)

Pathogenesis: deficiency leads to a sharp increase in the time of formation of the prothrombinase complex, which is accompanied by a long, almost does not stop bleeding with minor vessels trauma (biting tongue, bruises).

Manifestations:

It is characterized by hematoma type of bleeding. In mild form of disease the bleeding are possible only when significant trauma or surgical interventions, is subclinical and often not diagnosed. In severe or very severe form is manifested from newborns and first years of life in children. Recurrent bleeding in the large joints (hemarthrosis) lead to ankylosis. Large inter- and intramuscular, retroperitoneal hematoma with subsequent destruction of the soft tissues, severe and frequent spontaneous bleeding, persistent recurrent gastrointestinal bleeding and kidney.

Hemophilia B (Christmas disease)

Etiology: a deficiency of factor IX (Christmas factor). Inherited X-linked recessive.

Pathogenesis: defect leads to a significant slowdown in the formation of the prothrombinase complex that lead to the development of hematoma type of bleeding.

Manifestations:

The clinical picture is characterized by bleeding (hemarthrosis, hematomas), but their frequency is 5 times lower than in the deficiency of factor VIII.

Hemophilia C

Etiology: a deficiency of factor XI (plasma thromboplastin antecedent). Inherited as an autosomal recessive.

Pathogenesis: isolated violation of the internal mechanism of blood coagulation.

Manifestations:

In heterozygotes bleeding is minor.

Homozygotes have a few complications associated with bleeding, but the injury or surgery may cause severe bleeding and formation of hematoma and hemarthrosis.

Parahemophilia (Owren's disease)

Etiology: a deficiency of factor V (proaccelerin, labile factor, accelerator, globulin). Inherited as an autosomal recessive and autosomal dominant.

Manifestations

The disease is characterized by hemorrhagic syndrome: marked petechiae, ecchymoses, bruising, nasal, gingival, gastrointestinal bleeding, menorrhagia. In patients with severe forms of the disease is often prolonged bleeding after tooth extraction, trauma, cuts.

Female hemophilia

Female hemophilia meets very seldom (described 50 cases).It is a genetically heterogenous disease. The main variants of female hemophilia:

- normal chromotype (XX) and double inheritance of true hemophilia — appears in girls whose fathers have hemophilia and mothers are conductors of disease (often incest);
- normal chromotype (XX) and one sided hemophilia inheritance;
- incomplete chromotype with one X-chromosome (XO). These patient may have severe hemophilia form, as men from this family;
- in «women» with testicular feminization (XY);
- autosome-dominant forms of factor VIII deficiency (in this group should be excluded von Willebrand disease).

Von Willebrand disease

Etiology: Von Willebrand disease is the most common inherited disorder of bleeding (autosome-dominant type of inheritance). This disease is revealed in both sexes.

Pathogenesis: defetiency of Von Willebrand factor leads to the violation of thrombocytes adhesion to collagen of vascular wall and decrease the intensity of the formation of complex vWF-Factor VIII. In the absence of vWF, the factor VIII is subjected to accelerated destruction in the blood. A result is deficiency of factor VIII and related spontaneous hematoma bleeding. Thus, von Willebrand's disease characterized by impaired platelet and coagulation hemostasis.

Manifestations

Clinically it is manifested by bleedings into the skin and mucose membranes: nasal bleedings, ecchymoses, menorrhagies, hematuria, severe bleedings from a slight traumas or surgical wounds (in tonsillectomy, tooth extraction). Patients with von Willebrand disease have prolonged bleeding time with normal platelet count and also prolonged partial thromboplastin time.

Acquired coagulopathies

Disseminated intravascular coagulation syndrome (DIC syndrome)

DIC — nonspecific syndrome characterized by disseminated intravascular coagulation of blood proteins, aggregation of formed elements, activation and depletion of components coagulation and fibrinolytic systems, blockage of microcirculation blood vessels in organs followed microthrombogenesis.

Classification

By clinical course:

- acute;
- subacute;
- chronic.

By process localization:

- disseminated intravascular clotting (the organism level);
- localized intravascular clotting (in organ or part of organ limits).

By severity:

- compensated;
- subcompensated;
- decompensated.

Etiology:

- all kinds of shock, trauma (for example, crush syndrome);
- terminal states;
- obstetric syndromes (placenta, amniotic fluid embolism, atonic uterine bleeding, fetal death etc.);
- massive destruction and necrosis in organs (burns, frostbite, pancreatic necrosis, destructive pneumonia);
- traumatic surgery;
- acute intravascular hemolysis (transfusion of incompatible blood transfusion and massive) and chronic hemolysis (hemolytic anemia);
- severe infection and sepsis;
- persistent bacterial and viral infections;
- malignant tumors and hemoblastoses;
- dehydration;
- massive blood contact with a foreign surface (extracorporeal circulation, chronic hemodialysis);
- damage of the endothelium (dissecting aortic aneurysm, progressive atherosclerosis, systemic lupus erythematosus, rheumatoid arthritis, hemolytic uremic syndrome, acute glomerulonephritis, fever, allergic reactions).

Pathogenesis of DIC-syndrome:

- initial activation of coagulation system and thrombocytes by endogenous factors: tissue thromboplastin, leukocytic proteases, products of tissue destruction, tumoral procoagulants;
- long-lasting thrombinemia with the increased level of its markers in blood;
- depletion of anticoagulant with the decreased level of antithrombin III, protein C, plasminogen and the increased level of thrombomodulin;
- systemic endothelial injury and the decrease in its antithrombotic potential;
- formation of micro blood clots and block of microcirculation in target-organs (a brain, adrenal glands, a liver, kidneys, an intestine, a stomach) with dystrophy and destruction on these organs;
- activation of fibrinolysis in a zone of microcirculation block and its release exhaustion in blood circulation;
- consumption of coagulation factors and consumption thrombocytopenia (and thrombocytopathy), leading to bleeding and systemic hypocoagulation terminal until full incoagulability blood (hemorrhagic phase syndrome);

• violation of the barrier function of stomach and intestines mucous membrane with impairment of their barrier function and transformation of aseptic DIC-syndrome into the septic;

• secondary severe endogenous intoxication.

Stages of DIC-syndrome:

• hypercoagulation and thrombogenesis (short);

• consumptive coagulopathy (characterized by higher consumption and depletion of clotting factors and thrombocytes, hypofibrnogenemia and deficiency of anticoagulants);

• hypocoagulation (hypocoagulation-hemorrhagic phase) manifested by hemorrhagic syndrome;

• reparative (residual thrombosis and vascular blockade).

Manifestations:

DIC is characterized by symptoms of multiple organ failure as a result of injury and dysfunction of target organs:

1. Acute pulmonary insufficiency (up to pulmonary distress syndrome).
2. Acute renal or hepatorenal insufficiency (decreased urine output and uremia).
3. Cerebral symptoms associated with cerebral ischemia.
4. The damage of gastric and intestinal mucosa (heavy bleeding, acute hypoxic ulcers, impaired barrier function of the mucous membrane and transformation of aseptic DIC into the septic toxic form).
5. Adrenal or pluriglandular endocrine insufficiency hemodynamically unstable.
6. Systemic inflammatory response syndrome with accumulation of cytokines and other metabolites in the blood.

The main sub-syndromes observed in DIC:

Transformation of aseptic DIC-syndrome into septic. It is mostly associated with infection of the tissue damaged loci or barrier function impairment of intestinal mucose membrane with the subsequent massive entrance of bacterial population in blood. It is presented by fever, leukocytosis with nuclear shift to the left, the increased ESR, the inceased level of acute phase proteins (C reactive protein), interleukins. Participate in the development of terminal hemorrhagic syndrome.

Sub-syndrome of respiratory failure. Respiratory symptoms such as dyspnea, cyanosis and respiratory difficulty may dominate in the bleeding of DIC. Sub-syndrome of respiratory failure needs in controlled ventilation.

Sub-syndrome of acute renal and hepatorenel insufficiency. It is often needs in hemodialysis and plasmapheresis.

Sub-syndrome of the impairment and injury of other organs (adrenal glands, brain, heart) forms the poly-organic insufficiency syndrome.

Sub-syndrome of the stomach and intestine injury has three clinical manifestations: bleeding erosion and ulcer formation (shock or hypoxic ulcers), diffuse bleedings of mucosal membrane barrier function impairment with transformation of aseptic DIC into septic.

VASSOPATHY (ANGIOPATHY)

Vassopathies (angiopathies) are the diseases, which are characterized by the bleeding in the result of vascular wall pathology.

Etiology

Etiological factors may be exogenous (physical, chemical and biological) or endogenous (immunological, genetic), acquired and genetic.

The acquired forms develop as a result of ionizing radiation, deficiency of vitamin C (results in reduction of collagen synthesis), side effect of medicines, viral infection, various factors, which cause inflammation of vessels, including immune factors (autoimmune aggression against endothelium), disorder of vascular trophism, destruction of vascular walls as a secondary effect of leukemic infiltrates, disorder of the nervous and hormonal regulation of vascular tonus.

Genetic forms is a result of the mutations of genes, which are responsible for synthesis of endothelial proteins (enzymes, collagen, contractile proteins and other) and receptors

Pathogenesis

Damage of the vessels under exogenous and endogenous factors results in inflammation and impairment of endothelium participation in hemostasis (hemorrhagic vasculitis). Endothelium loses ability for production of active substances (prostaglandins, procoagulants) with results of disorders of the thrombocyte-vascular hemostasis. There are occurs predisposition to bleeding.

Congenital angiopathies are presented by telangiectasia (arteriovenous aneurysms with subendothelial maldevelopment and lack of collagen), hemangioma (vascular tumor).

THROMBOPHILIC STATE

Thombophilia is an inherited or acquired disorders of hemostasis mechanism predisposing to early emergence and recurrence of thrombosis and obliteration of blood vessels, ischemia and infarctions of organs.

Thrombophilia is divided into a number of major groups:

- hemorheological form (hemoconcentration, increased viscosity (dehydration, hyperfibrinogenemia paraproteinemia), Ht, Hb, RBCs (polycythemia vera), changes in the shape and deformability of RBCs (heredity hemolytic anemias);
- vascular and thrombocyte disorders of hemostasis due to:

✓ significant increase in thrombocytes level in blood (up 1200×10^9/l);

✓ increase in adhesiveness and aggregation of thrombocytes (viscous platelets syndrome, atherosclerosis, diabetes mellitus, taking hormonal contraceptives, etc.);

✓ overproduction of vWF;

- hereditary or acquired deficiency or abnormalities of anticoagulants;
- overproduction or hereditary abnormal plasma coagulation factors (factors lose their sensitivity to physiological anticoagulant or fibrinolytic system components);
- genetically or acquired disorders of fibrinolysis (decreased tissue plasminogen activator or increased plasma levels of its inhibitors, deficiency or abnormality of plasminogen);
- thrombophilia with metabolic origin (decrease in antithrombotic potential of endothelium and complex disorders in all links of hemostatic system: in atherosclerosis, hyperlipidemia, hyperhomocysteinemia, diabetic angiopathy etc.);
- autoimmune and infectious-immune thrombophilia (antiphospholipid syndrome);
- paraneoplastic thromboembolic syndromes **(**thrombotic complications in all cancer types (visceral forms) in surgical operations and chemiotherapy);
- iatrogenic (medical) forms (in catheterization and surgical operations of heart and vessels, vessel and valve replacement, in bone marrow stem cell transplantation, drug-induced forms);
- complex forms of thrombophilia (presents two or more impairments).

The most known forms of thrombophilia are hypermonocysteinemia and antiphospholipid syndrome.

SYNDROME IN HEMOSTATIC DISORDERS

Violation of the hemostatic system is characterized by hemorrhagic and thrombotic syndromes.

Hemorrhagic (manifested by bleeding)

Distinguish the following types bleeding:

Capillary or microcirculatoric (pethechia-bruise) type of hemorrhage. It is characterized by the appearance of small painless pointed or spotted hemorrhagies on the skin. Hemorrhages are often associated with menorrhagias, gum bleeding, rarely with the retina hemorrhage and gastric bleedings. Hemorrhages are easily provoked by mechanical traumas of microvessels. This hemorrhage type is characteristic of thrombocytopenia and thrombocytopathia, von Willebrand disease, prothrombin complex factor insufficiency (VII, X, V, II), some hypo- and disfibrinogenemias, moderate overdosage of anticoagulants. The bruise type of hemorrhage is often admitted in hereditary thrombocytopathias; the pethechial type of hemorrhage is not characteristic.

Hematoma. It is characterized by painful strained hemorrhages in subcutaneous cellular tissue, muscles, large joints, in peritoneum and retroperitoneal space. It may lead to nerve compression, joint destruction, bone tissue destruction, locomotor apparatus impairments. Renal and gastro-intestinal bleedings may develop.

Prolonged bleedings in wounds, tooth extractions, surgical operations may develop. It is observed in hereditary blood coagulation impairments (hemophilia A, B, excess insufficiency of factor VII), in hereditary thrombocytopathia with absence ofthrombocytic factor 3, acquired coagulopathias associated with appearance of factors VIII, IX, VIII + V inhibitors in blood, in overdosage of anticoagulants.

Complex capillary-hematoma type of bleeding. It is characterized by pethechial-bruise hemorrhages associated with numerous dense hemorrhages and hematomas. In the absence of joint and bone injury (unlike hematoma), bruises may be large and painful. This type of hemorrhage is observed in hereditary (excess insufficiency of VII and VIII factors, severe form of von Willebrand disease) and acquired impairments (acute, subacute DIC-syndrome, overdosage of direct and non-direct anticoagulants).

***Vasculitis purple type of hemorrhage*.** It is characterized by the appearance of monomorphis papulous-hemorrhage on palpation. The eruption elements do not disappear on touch. Nephritis development and intestinal bleedings are possible. This type of hemorrhage is observed in infectious and immune vasculites.

Angiomatous hemorrhage. It is characterized by recurring local bleedings. It is observed in angiomas, arterial venular anastamoses, teleangioectasias (Rendu-Osler disease). Bleeding is associated with slight injury of vascular wall in angioectasia loci and decreased stimulation of adhesion and aggregation of thrombocytes in this area. There are three types of teleangioectasias: early — small irregular form spots; intermedial – vascular spiders; late (nodular) — bright red nodes 5–7 mm.

Thrombotic (manifested by thrombosis of different localization)

Develops in the presence of the Virchow triad:

1. Damage of vascular wall and of endothelium loss of thromboresistance.
2. The imbalance between the activity of coagulation, anticoagulation and fibrinolytic systems.
3. Slowing blood flow.

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