

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ  
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ  
КАФЕДРА ПАТОЛОГИЧЕСКОЙ ФИЗИОЛОГИИ

# ПАТОЛОГИЧЕСКАЯ ФИЗИОЛОГИЯ СИСТЕМЫ КРОВИ

## PATHOLOGICAL PHYSIOLOGY OF THE BLOOD SYSTEM

Учебно-методическое пособие



Минск БГМУ 2010

УДК 616–092.18 (811.111)  
ББК 52.52 (81.2 Англ – 923) я 73  
П 20

Рекомендовано Научно-методическим советом университета в качестве  
учебно-методического пособия 28.04.2010 г., протокол № 9

**А в т о р ы:** д-р мед. наук, проф. Е. В. Леонова; канд. мед. наук, доц. А. В. Чантурия; д-р мед. наук, проф. Ф. И. Висмонт; канд. биол. наук, доц. С. А. Жадан

**Р е ц е н з е н т ы:** д-р мед. наук, проф. каф. морфологии человека А. А. Артишевский; канд. мед. наук, доц. каф. нормальной физиологии А. Н. Харламова

Перевод на английский язык Т. Ф. Даниловой, С. А. Жадан

**Патологическая физиология системы крови = Pathological physiology of the blood system** : учеб. метод пособие / Е. В. Леонова [и др.] ; пер. на англ. яз. Т. Ф. Данилова, С. А. Жадан. – Минск : БГМУ, 2010. – 64 с.

ISBN 978–985–528–185–7.

Приводятся современные сведения о кроветворении, нарушениях процессов эритро-, лейко-, тромбоцитопоэза. Рассматриваются типовые виды и реактивные изменения общего объема крови, систем форменных элементов крови, вопросы, касающиеся этиологии и патогенеза анемий, эритроцитозов, лейкозов, гемостазиопатий, их гематологической картины.

Предназначается для студентов 3-го курса факультета иностранных учащихся.

УДК 616–092.18 (811.111) (076.5)  
ББК 52.52 (81.2 Англ – 923) я 73

ISBN 978–985–528–185–7

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медицинский университет, 2010

## INTRODUCTION

The blood is one of the most important vital systems of the organism.

The specificity of the blood system is particularly important due to the fact that its pathologic changes occur as a result not only of functional impairment of its separate components but also of other organs and systems of the organism as a whole. Any disease, pathologic process as well as a number of physiological shifts may affect some qualitative and quantitative peculiarities of the circulating blood content to this or that degree. It is this that determines great significance of the necessity to study the blood (as «a blood mirror of the organism») and to determine the laws of its changes in various diseases.

The impairments in the blood system are revealed in typical forms of pathology and reactive changes in:

- the total volume, the ratio of plasma and blood corpuscular elements;
- system of erythrocytes;
- system of leukocytes;
- system of thrombocytes;
- system of hemostasis.

## Chapter 1

### TYPICAL FORMS OF PATHOLOGY AND REACTIVE CHANGES OF THE TOTAL VOLUME, THE RATIO OF THE PLASMA AND BLOOD CORPUSCULAR ELEMENTS

The total blood volume in an adult comprises 5–8 % of the body mass, i. e. on an average 4.5–5.1. The corpuscular elements comprise on an average 36–48 % of the total blood volume (hematocrit or the hematocrit factor — the ratio of the corpuscular elements volume to the plasma volume; in men it ranges 40–48 %, in women — 36–42 %).

Both the total blood volume and the ratio of corpuscular elements and plasma may change under the conditions of pathology. There are three basic groups of typical forms of impairments.

#### *Normovolemia*

Normovolemia (from Latin — norma-pattern + French volumen-volume + Greek haima-blood) is the state when a normal blood volume is preserved, but the ratio of corpuscular elements and plasma changes. There are:

1. Simple normovolemia — is the state when a normal blood volume and a normal ratio of corpuscular elements and plasma take place;

2. Oligocytic normovolemia (hemodilution) is characterized by a normal blood volume and a decrease of the count of corpuscular elements (mainly of erythrocytes) that is accompanied by a drop of hematocrit below 36 %.

3. It occurs in massive hemolysis of erythrocytes, suppression of hemopoiesis, after acute loss of blood, when the blood volume comes quickly to the norm at the expense of the tissue fluid entering vessels, while the erythrocyte count still remains decreased. This state is manifested by hypoxia. A considerable decrease of the erythrocyte count may cause slowing down of blood coagulation and a hemorrhagic syndrome, while a prolonged decrease of the leukocyte count — a decrease of anti-inflammatory and anti-tumor resistance.

4. Polycytemic normovolemia (hemoconcentration) is characterized by a normal total blood volume, while the corpuscular elements count exceeds 48 %. It may be caused by chronic hypoxic conditions. It is manifested by the impairment of microcirculation due to blood thickening, an increase of its viscosity, thrombus formation slowing down the blood flow, decrease of intensity of transcapillary exchange. In considerable polycytemia, arterial hypertension may develop.

#### *Hypervolemia*

Hypervolemia (from Greek. hyper — over, exceeding the norm + volemia) — is the condition characterized by an increase of the total blood volume and most often by the impairment of the ratio of corpuscular elements and plasma.

1. Simple (normocytic) hypervolemia — is an increase of the total blood volume, while a normal per cent ratio of the plasma and corpuscular elements is preserved. It takes place during a short period of time in transfusing great amounts of donor blood, on great physical exertions, in acute hypoxia, when environmental high temperature comes into effect, when deponed blood comes into the blood stream from the depot and interstitial fluid from tissues. Such condition may result in a decrease of the vascular tone, cardiac overloading, development of cardiac insufficiency.

2. Oligocytic hypervolemia (hydremia) — an increase of the blood volume at the expense of mainly a fluid part, the hematocrit factor being below 36 %. It occurs in the impairment of the excretory function and retention of fluid in the blood stream, pathologic thirst, excessive injection of physiologic solution or blood substituting solutions, in hyperproduction of an anti-diuretic hormone. As a result the blood circulation impairment may occur due to overstretching of vessels, cardiac cavities and microcirculation impairment.

3. Polycytic hypervolemia — is the condition, when the circulating blood volume increases mainly at the expense of corpuscular elements (erythrocytes) due to which the hematocrit factor exceeds 48 %. It occurs in heart defects, chronic circulation insufficiency, alveolar hypoventilation, decrease of oxygen blood capacity and efficiency of biologic oxidation, exogenous (hypo- and normobaric) hypoxia, as well as in erythremia (Vaquez' disease) — leucosis with predominant damaging of the red marrow germ (see below). The disease is accompanied by an increase of blood viscosity, arterial blood pressure, increase of the cardiac loading followed by hypertrophy of the left ventricle, etc.

### ***Hypovolemia***

Hypovolemia (from Greek. hypo — supra, below the norm + volemia) — is the condition characterized by a decrease of the total blood volume and impairment of the ratio of corpuscular elements and plasma.

1. In the majority of cases simple (normocytic) hypovolemia is characterized by a decrease of CBV (circulating blood volume) in normal hematocrit. Its causes are acute hemorrhages, shock conditions, a vasodilatational collapse. In the last two cases there occurs deponing of a considerable amount of blood in venous (voluminous) vessels and a considerable decrease of CBV. The danger of this condition includes a decrease of arterial pressure, impairment of peripheral blood flow causing hypoxia and the impairment of tissue metabolism.

2. Oligocytic hypovolemia is characterized by a decrease of the total blood volume with a predominant decrease of corpuscular elements and hematocrit below 36 %. It is observed immediately after the blood loss, when its migration from the depot and tissue fluid hasn't eliminated hypovolemia yet and the outlet of blood cells from hemopoietic organs — deficiency of erythrocytes, as well as in massive hemolysis of erythrocytes or suppression of their produc-

tion in bone marrow. It is manifested by the disturbance of blood circulation in various vessels, decrease of the blood oxygen capacity due to erythropenia.

3. Polycytemic hypovolemia (anhydremia) is observed in decreasing of the total blood volume due to a predominant decrease of the plasma volume, the hematocrit volume exceeding the normal one. The most frequent causes of this condition are various forms of dehydration, pernicious vomiting, profuse diarrhea, polyuria, intense perspiration, expansive burns, water fasting, hyperthermia, diabetes incipidus, etc. There are observed disturbances of the central, organ-tissue and microhemocirculation systems.

The greatest clinical significance of all mentioned conditions has a blood loss.

### **1.1. BLOOD LOSS**

Blood loss — is a pathological condition as a result of losing a part of blood (hemorrhage) leading to disturbances of vital activity of the organism to a various degree. Hemorrhages can be caused by: 1) rupture of a vessel (a mechanic lesion) — hemorrhagia per rhexin; 2) destruction of the vascular wall by a pathologic process (gastric ulcer, tumor, atherosclerosis of large vessels) — hemorrhagia per diapedesin; 3) increasing the permeability of the vascular wall (radiation disease, hematosarcoma, extramedular foci of hemopoiesis, some infectious processes) — hemorrhagia per diapedesin.

The character of the course and outcome of the blood loss are determined by the following factors:

1. The volume of lost blood. The blood loss up to 15–22 % of CBV is light, of small danger, and is compensated by triggering urgent compensation mechanisms. The blood loss up to 25–35 % of CBV (moderate severity) involves marked disturbances of the central, organ-tissue and microhemocirculation. A severe degree develops in the loss of 50 % and over of the total blood volume, it may be lethal.

2. The hemorrhage velocity. The less it is, the less marked are the disturbances of vital activity. A sudden acute loss of 50 % of blood is lethal, while a moderate (within some days) loss of the same blood loss may avoid a fatal outcome as there is time for triggering adaptation reactions. Acute blood loss up to 25–50 % of CBV are considered to be threatening to life and may result in the development of hemorrhagic shock.

3. Reactivity of the organism (age, sex, type of higher nervous activity, functional state of large hemispheres at the moment of hemorrhage, the ratio of the coagulating and anti-coagulating blood systems, etc.).

The amount of a fatal blood loss is relative. It may be both greater (60–70 % of CBV) and less (15–20 %) depending on peculiarities of the organism reactivity. Experimental investigations have shown that for dogs exposed to preliminary heating or cooling a lethal loss proved to be only 15 % of blood.

The resistance to a blood loss is reduced also in the state of deep narcosis, in pain stimulus. The combination of mentioned stimuli with a blood loss may prove to be excessively strong for the central nervous system, to result in fast exhaustion of cortical cells of the brain large hemispheres and subcortical centers. Women are less sensitive to a blood loss, adult persons endure it better than children; a recurrent loss of small volumes of blood may produce a «training» effect, enhance the resistance of the organism to a blood loss.

Changes in the organism in blood loss are presumably divided into three stages: an **initial**, a **compensation stage** and a **terminal** one.

The **initial stage** is characterized by a decrease of CBV, the development of simple hypovolemia, the in-flow of the venous blood to the heart, a stroke and minute output of the blood by the heart, a drop of the arterial pressure level, vascular perfusion pressure in organs and tissues, the development of capillary-trophic insufficiency, circulatory hypoxia, impairment of energetic and plastic supply of cells, the vital activity of the organism is disturbed.

The described changes are a signal for triggering and activating protective-adaptation reactions and transitions of the process to the **second (compensatory) stage**. There are immediate and delayed mechanisms of compensation. Immediately after an acute blood loss, on the background of the resulted stress, urgent hemodynamic mechanisms of compensation are triggered. Due to irritation of receptor vascular zones, the tone enhancement of the sympathetic nervous system, output of catecholamines by adrenal glands, there occurs a reflex spasm of small arteries and arterioles, the vascular resistance of internal organs increases (except the brain and the heart) and the skin, the blood supply of the skin, muscles, internal organs decreases that contributes to sustaining of the blood flow to the heart and in the brain (centralization of blood circulation).

There occurs migration of blood into the blood stream from the depot followed by elevation of arterial blood pressure and partial restoration of CBV. Due to activation under hypoxia of the sympato-adrenal system and decrease of the cardiac output there occurs a reflex increase of the rate and intensity of cardiac contractions that partially increase the cardiac output as well as reflex acceleration and deepening of respiration contributing to elimination of oxygen deficiency in the organism. Due to enhancement of dissociation of oxyhemoglobin in developed acidosis the ability of hemoglobin to adjoin oxygen and give it to tissues is increased as well as the factor of oxygen utilization.

Alongside with hemodynamic compensation, hydramic compensation is triggered. In posterior nuclei of the hypothalamus CBV reduction activates the synthesis and incretion of the factor stimulating the production of aldosterone in the glomerular zone of adrenal glands resulting in activation of Na-ion reabsorption in distal parts or renal canaliculi and elevation of the osmotic pressure of blood plasma (volume-reflex). Hyperosmia of the blood «triggers» an osmoreflex: excitation of osmoreceptors of the blood channel activates

the neurosecretion of ADH in the hypothalamus, its transport to the posterior lobe of the hypophysis and then into the blood. ADH increases permeability of the walls of renal canaliculi for fluid, and it enters into the blood on the gradient of osmotic pressure (hyponatremia). Simultaneously, on the gradient of osmotic pressure, the flow of fluid from the cells passes into the interstitial space and then into lymphatic capillaries and into the blood (autohemodilution). There occurs blood dilution and increase of CBV (oligocytemic normovolemia or hypovolemia). The hemostasis system is activated, it is revealed by accelerating blood coagulation contributing to cessation of bleeding. The vascular wall lesion observed in bleeding is accompanied by activation of thrombocyte and plasma components of hemostasis, while a decrease of arterial pressure may cause the arrest of the peripheral blood flow, blood stasis in the system of microcirculation followed by the development of a DBC-syndrome.

Immediate compensation mechanisms are revealed later. They include activation of erythropoiesis under the effect of increased erythropoietine production. On the 4<sup>th</sup>–5<sup>th</sup> day after the hemorrhage the peripheral blood reveals regenerative forms of erythrocytes (see below), proliferation and maturation of cells of a lymphocyte and thrombocyte germ of hemopoiesis are also stimulated (medullar compensation). The protein composition of blood starts increasing in 2–3 days after the hemorrhage due to mobilizing tissue resources, but its normalization occurs on the 8<sup>th</sup>–10<sup>th</sup> day due to activation of proteins synthesis in the liver (protein compensation).

The **terminal stage** of blood loss may occur in insufficiency of adaptation reactions associated with severe diseases under the effect of unfavorable exogenous and endogenous factors, expansive injury, acute massive blood loss exceeding 50–60 % of CBV and the absence of treatment. Resulting pathologic changes are revealed alongside with a general anemic syndrome such as pallor, weakness, coolness of the skin, breathlessness, acceleration of HR (up to 140–150 per min), decrease of arterial pressure, weak pulse, yawning, a feeling of fear, general depression, pupils dilation, dullness and loss of consciousness, twitching of muscles, involuntary urination and defecation, appearance of arrhythmias and other impairments of the most important functions of the organism. Death in blood loss occurs from paralysis of the respiratory center sometimes accompanied by simultaneous heart arrest.



## Chapter 2 HEMOPOIESIS, GENERAL LAWS

Blood formation (hemopoiesis) — is the process, when a series of cellular differentiations occurs; it is followed by the formation of mature cells of the peripheral blood taking place in hemopoietic organs.

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

*The yoke (mesoblast, angioblast) period* starts on the 2<sup>nd</sup>–3<sup>rd</sup> week of the antenatal life, the primary primitive erythroblasts — megaloblasts (megaloblastic erythropoiesis) being formed in vessels of the yoke sac, and by the end of the period the first elements of a normoblastic series and white blood (extravascularly) appear.

On the 2<sup>nd</sup> month (after the 6<sup>th</sup> week) the second period starts — *hepatic*. Blood formation occurs in the liver and thymus extravascularly on megaloblast, normo-, myelo-, lympho-, monoblast and megakaryoblast types.

By the end of the 4<sup>th</sup> month the megaloblast type of hemopoiesis disappears gradually. The 3<sup>rd</sup> one starts — *a medullary (myeloid) period*.

Blood formation is accomplished extravascularly in the red bone marrow, lymphatic glands, thymus, spleen, lymphoid tissue of the intestines. Erythrocytes are formed on a normoblast type, granulocytes (neutrophiles, eosinophiles, basophiles) — on a myeloblast type, lymphocytes — on a lymphoblast type, monocytes — on a monoblast type, thrombocytes — on a megakaryoblast type of blood formation.

In postnatal life the bone marrow becomes a basic hemopoietic organ. The intensity of hemopoiesis in the rest of the organs quickly decreases after birth.

The progenitor of all cells of the blood system is polypotent stem hemopoietic cells — PSHCs, comprising *the first class of hemopoietic cells*. PSHCs — are morphologically unrecognizable; they may be identified by immune-morphologic methods. Antigen CD34 is a marker of these cells.

*The second class cells* — are polypotent progenitor cells — colony-forming units. Under the effect of the colony-stimulating factor of stem cells (CSF), interleukines IL-1, IL-6 a PSHC transforms into a semi-stem (multipotent) PSHCs progenitor cell of lymphopoiesis (CFU-L, and under the effect of CSF, IL-1, IL-3, IL-6 and granulocyte colony-stimulating factor (GCSF) — into a cell-progenitor of myelopoiesis (CFU-GEMM), as well as into a semi-stem multipotent cell.

*The third class* involves biopotent progenitor cells differentiated by two germs. They form large colonies-bursts (BFU) or smaller, more mature colonies (CFU). These cells are not capable of prolonged self-sustaining, they intensely proliferate and differentiate. A progenitor-cell of lymphopoiesis, a pre-T lymphocyte, gives the start to T-lymphocytes, while a pre-B lymphocyte —

to B-lymphocytes. A progenitor cell of myelopoiesis (CFU- GEMM) may give three differentiation series of colony-forming units:

- eosinophile (CFU-Eo), basophile (CFU-B), granulocyte — neutrophile (CFU-G), monocyte (CFU-M), and erythroid (CFU-E) series;
- granulocyte-monocyte series (CFU-GM);
- Erythrocyte-megakaryocyte (CFU-EMk) series.

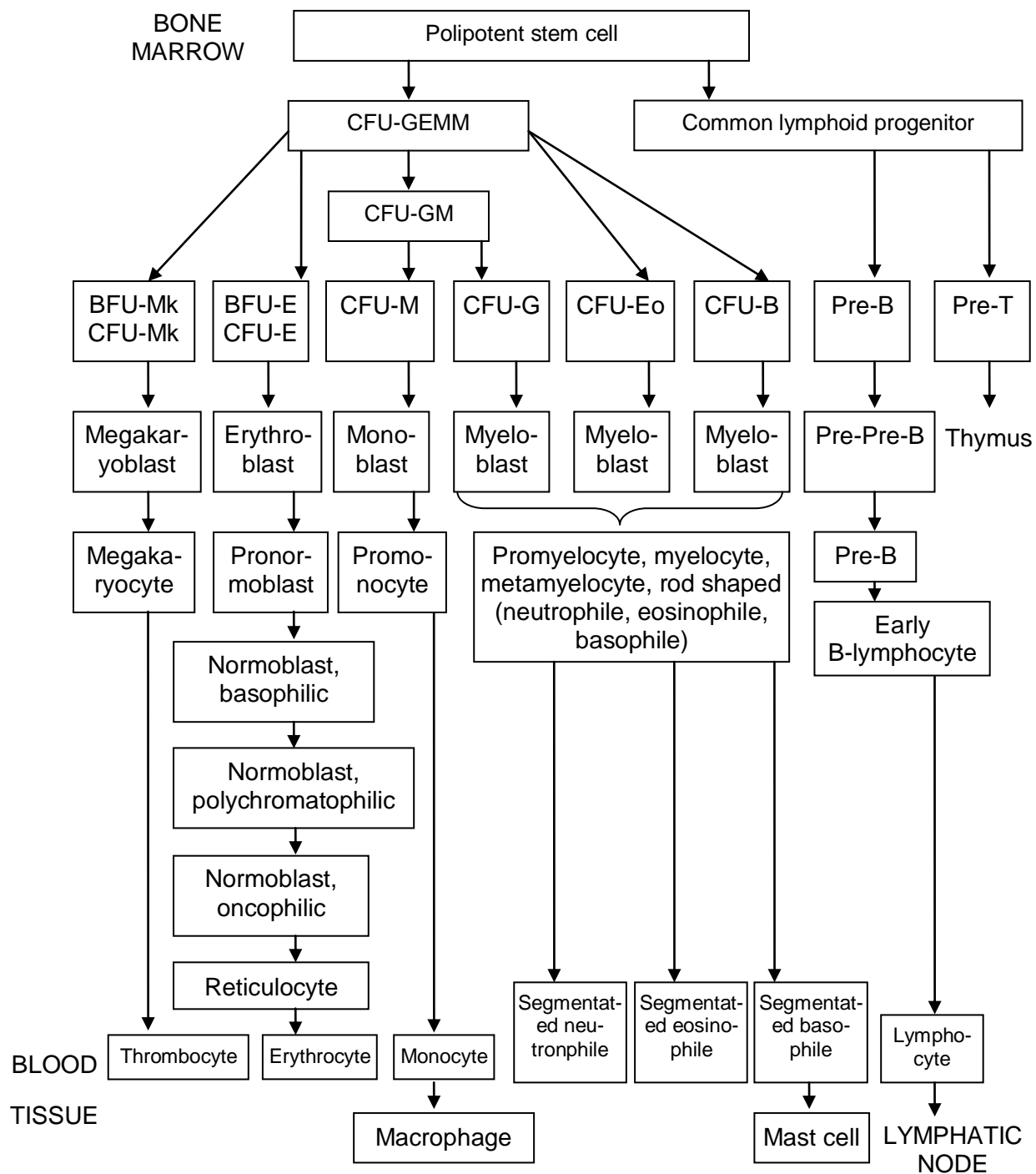
Differentiation of all progenitor cells is accomplished under the effect of growth factors specific for every series.

Having performed a number of mitoses the *3<sup>rd</sup> class cells* transform into the *4<sup>th</sup> class cells* — unipotent progenitor cells specific for every hemopoietic line. They are not self-sustaining and after division they differentiate and transfer into the *5<sup>th</sup> class cells* — morphologically identified cells presented by lympho-myelo-erythro-megakaryoblasts. The latter differentiate towards one definite cellular series and differ morphologically, immune-phenotypically and cytochemically.

*The cells of the 6<sup>th</sup> and 7<sup>th</sup> series* comprise accordingly maturing and mature specifically functioning cells of hemopoietic organs and the peripheral blood of some hemopoietic germs. They are highly differentiated cells with a short life span, incapable of proliferation and differentiation in other directions (diagram).

Pathology of hemopoiesis may be manifested by:

- the impairment of cellular maturation;
- the entrance of immature cellular elements into the blood;
- the appearance of cellular elements in the peripheral blood that are uncharacteristic of this age category.



Medullary hemopoiesis

## Chapter 3

# THE SYSTEM OF ERYTHROCYTES (ERYTHRONE) AND ITS IMPAIRMENTS

The whole mass of erythroid cells of the organism is united by the notion of «erythrone». It includes progenitor, proliferating, maturing, mature, specifically functioning and destroyed cells, presents a functional system performing a highly specialized gas-transporting function that contributes to the production and sustaining, at a rather high level, of the whole mass of erythrocytes containing hemoglobin and providing tissues with oxygen.

### 3.1. ERYTHROPOIESIS

Erythropoiesis — is a process of structural, metabolic and functional differentiation that starts with the formation a polipotent stem cell and completes with the formation of a mature erythrocyte. There are megaloblast- and erythroblast types of hemopoiesis.

**Megaloblast erythropoiesis.** The first morphologically identified cell of this series — a promegaloblast. It is rounded or irregular in shape (25–30  $\mu\text{m}$ ). The cytoplasm is basophilic (does not contain *Hb*). The nucleus occupies the greater part of the cell, is round or oval with a delicate net of chromatin, is stained in a red-violet color and has 2–5 nucleoli.

Then a *basophilic megaloblast* is formed (20–30  $\mu\text{m}$ ). The nucleus occupies  $\frac{2}{3}$  of the cell, is located eccentrically in most cases, has a delicate net of chromatin, is stained in a violet or red-violet color.

Then a *polichromatophilic megaloblast* appears (16–25  $\mu\text{m}$ ). The cytoplasm is stained in a grey-lilac (grey-pink) color. The nucleus is the same as a basophilic megaloblast or more compact.

Then an *oxiphilic megaloblast* is formed — oval in shape. The cytoplasm is intensive-pink. The nucleus is compact, pyknotic, dark-violet, eccentrically located.

A *megalocyte* is formed on the last stage (12–15  $\mu\text{m}$ ) — a cell without the nucleus of oval or irregular shape, without clearance in the center, contains a lot of hemoglobin (*HbF*) and that is why is stained in an intensive pink color. A long life span of a megalocyte is 2–3 weeks; this cell easily undergoes hemolysis. Megaloblasts are not capable of transforming into a normal erythrocyte. Only their insignificant part transforms into megalocytes entering the circulating blood.

**Erythroblast (normoblast) erythropoiesis.** The progenitor of erythrocytes only is a unipotent burst-forming unit of the bone marrow (BFU). A more mature form is a unit forming a less numerous erythroid colony — CFU. Under the influence of erythropoietine affecting the superficial receptors of erythroid cells the latter transform into erythroblasts.

An *erythroblast* (15–25  $\mu\text{m}$ ) — the first morphologically recognizable cell of a normoblastic series. The cytoplasm is dark-blue with a prenuclear zone of clearance. The nucleus has a delicate net of chromatine, contains 1–3 nucleoli, occupies the most part of the cell and is stained in a red-violet color. The next stage of development — a *pronormoblast* (*pronormocyte*) — 12–18  $\mu\text{m}$ , the cytoplasm is basophilic, the nucleus of a red-violet color, less in size, with a rough structure, does not contain any nucleoli. This cell gradually transforms into a *basophilic normoblast* (*normocyte*) (10–18  $\mu\text{m}$ ), the nucleus is still less, with a rough radial (wheel-like) structure. Then a *polychromatophilic normoblast* (*normocyte*) (9–12  $\mu\text{m}$ ) is formed. The cytoplasm is stained in a grey-lilac (grey-pink) color (takes both acid and base stains). The nucleus with a wheel-like structure and signs of pyknosis. An *oxiphilic normoblast* (*normocyte*) appears on the next stage — 7–10  $\mu\text{m}$ , the nucleus is dense, roughly pyknotic («cherry seed», «ink blot»), is stained in a dark-violet color.

Pushing out the nucleus on this stage the cell transforms into an *erythrocyte*, that always preserves the remains of basophilia due to a small amount of RNA disappearing within the first day. Such a young erythrocyte with remains of basophilia is called a polychromatophile (a grey-lilac erythrocyte 9–11  $\mu\text{m}$ ). When special life-time staining is used (brilliant cresyl blue), the cell acquires a light bluish-dark bluish color and a basophil substance is revealed as a net, filaments, granules (*substantia granulo-reticulo-filamentosa*). Then this cell is called a reticulocyte.

A mature erythrocyte (7–8  $\mu\text{m}$ , 1.5–2 times less than a megalocyte) presents a concavo-concave, discoid cell without the nucleus, is stained in a pink color with clearance in the center, contains *HbA*. The life span of an erythrocyte is 100–120 days.

### 3.2. STRUCTURAL-FUNCTIONAL CHARACTERISTIC OF AN ERYTHRONE IN NORM AND PATHOLOGY

The erythropoietic tissue of the human organism occupies 20–30 % of the bone marrow. Under normal conditions the blood cells of the first IV classes are in hemopoietic organs, while the cells of the VII class — in the peripheral blood. They can be undeponed (be in the circulating peripheral blood) and deponed (be in the blood depot).

When life conditions change, general erythropoiesis increases or decreases depending on the needs of the organism in erythrocytes at the moment. The number of erythroid cells maturing to the stage of an erythrocyte characterize the degree of effective erythropoiesis, while the production of functionally defective erythrocytes and the process of intracerebral destruction of erythroid nucleus-containing cells is defined as *ineffective erythropoiesis*.

An erythrocyte is a specialized cell of the peripheral blood, containing the most important respiratory pigment *Hb* and providing oxygen supply from

pulmonary alveoli to all cells of the body and of carbon dioxide from cells to the lungs. Thanks to the shape of erythrocytes they are characterized by a high ratio of the surface and volume due to which any molecule of *Hb* there is located close to the surface providing maximally accelerated gas exchange.

Ageing of erythrocytes is associated with a decrease of activity of their enzyme systems. Since the 60<sup>th</sup> day after entering of erythrocytes into the peripheral blood there is observed a progressive decrease of activity of glucose-6-phosphatkinase and other enzymes resulting in a decrease of the energetic potential of the cell. At the end of its life cycle erythrocytes are characterized by their smaller sizes, greater concentration of hemoglobin, expression of a specific glycoprotein-antigen not characteristic of young and mature cells, unspecific antigen of ageing cells (AAC). The appearance of AAC serves a «signal» for ontogenetically programmed elimination of corpuscular elements, that have become old, resulting in immune response (physiological antibodies to AAC are permanently present in the blood serum in small titers). Old erythrocytes undergo immune-mediated hemolysis and phagocytosis. To destruction (erythrodiuresis) are exposed not only ageing erythrocytes, but also a part of nucleus-containing cells of the bone marrow (intramarrow ineffective erythropoiesis), functionally defective erythrocytes that have entered the peripheral blood (a peripheral component of ineffective erythropoiesis). Under normal conditions erythrodiuresis occurs inside mononuclear phagocytes. Defective erythrocytes undergo diuresis in the spleen. Hemoglobin comprises about 95 % of the protein of erythrocytes. It refers to complex proteins-chromoproteids. It contains an iron-bearing prosthetic group — hem (4 %) and simple protein of albumin-type — globin (96 %). The synthesis of *Hb* occurs on early development stages of erythroblasts. Hem — is an active group of hemoglobin, starts its active synthesis later on. The synthesis of globin and hema occurs in erythroid cells independently of each other. The blood of an adult person in norm contains three types of hemoglobin: *HbA* (96–98 %); *HbA<sub>2</sub>* (2–3 %) and *HbF* (1–2 %). On early stages of embryogenesis (from the 19<sup>th</sup> day to the 6<sup>th</sup> week) embryonic hemoglobins are mainly synthesized: Gower-1, Gower-2 и Portlad-primary embryonic — HBP (primitive).

During the specified period the blood formation gradually switches from the yoke sac to the liver. By the 4<sup>th</sup> month erythrocytes of a hepatic origin dominate in the circulating blood and contain fetal hemoglobin *F* (Hb F — foetal).

Hemoglobins differ in biochemical, physical-chemical, immunobiological properties. Thus, *HbF* as compared to *HbA*, possesses a higher affinity to oxygen and is capable of faster releasing carbonic acid. Thanks to these peculiarities the tissues of the fetus and child are supplied by oxygen under various conditions of their existence. By the moment of birth the child has both types of *Hb* (*HbF* u *HbA*). Then the «uterine» *Hb* is gradually changed for the «adult» *Hb* and by the end of the 2<sup>nd</sup> year of life it usually disappears. Sometimes there

may be revealed a minimum (up to 2 %) amount of *HbF* that has no pathologic significance.

In mutations abnormal hemoglobins are formed in polypeptide chains of globin of structural genes controlling *Hb* synthesis, when aminoacids are changed.

Over 400 abnormal *Hb* are known with characteristic impairments of the primary structure of this or that polypeptide chain of *HbA* (hemoglobinopathies or hemogloninoses). The basic types of such *Hb* are:

- sickle-cell hemoglobin (*HbS*) — occurs in substitution of glutamine acid for valin in  $\beta$ -chain; in this case a sickle-cell anemia develops (see below);

- methemoglobins (about 5 varieties) are formed, if histidine is replaced for tyrosine; in this case oxidation of *Hb* into methemoglobin, permanently taking place in norm, becomes irreversible, which is not characteristic of a healthy person;

- hemoglobins revealing weak affinity to oxygen, intensive supply of oxygen to tissues, repression of erythropoietine production and causing anemia;

- hemoglobins, revealing high affinity to oxygen cause the development of a dominant polycytomia, as the decrease of oxygen supply to tissues causes hypoxia followed by a compensatory increase of erythropoietine production, etc.;

The carriers of mentioned abnormalities number about  $100 \cdot 10^6$  individuals all over the world.

A great number of factors take part in regulation of erythropoiesis. By their final effect they are divided into stimulators and inhibitors of erythropoiesis.

Among stimulators of erythropoiesis the main part plays erythropoietine (EPO) — the main physiologic stimulator of erythropoiesis. Erythropoietine — is a glycoproteid, in the fetus it is formed in the liver, where its synthesis is preserved in a minimum volume after birth. After birth it is synthesized mainly in kidneys. Basic stimulators of erythropoietine formation are hypoxia, androgens, hemolysis products, carbon monoxide. In chronic renal diseases, nephrosclerosis, after hypophysectomy, hypopituitarism the level of EPO decreases, that underlies the pathogenesis of corresponding anemias.

The main physiologic inhibitor of erythropoiesis is an erythrocyte keilon, secreted from mature erythrocytes. Simultaneously an erythrocyte antikeilon also exists, which stimulates the entrance of dividing cells into a synthesis phase of DNA.

Erythropoiesis depends on the whole group of metabolic factors, vitamins and trace elements. The most important of them are:

- vitamins  $B_{12}$  and folic acid; internal antianemic Castle's factor — hexosamine, containing mucoprotein of parietal cells of the fundal part of gastric mucous membrane; with vitamin  $B_{12}$  (external factor) it forms a complex, pro-

protecting vitamin B<sub>12</sub> from destruction in the intestines; ferrum — a component of the active center of hemoglobin, necessary for synthesis of hemoglobin; copper — is necessary for erythropoiesis, participates in stimulation of reticulocytes maturation, activating cytochromoxidase of hemopoietic cells.

A definite significance for cellular division and protein synthesis in an erythron have nickel and cobalt (components of vitamin B<sub>12</sub>), molybdenum (is contained in enzymes, providing some stages of purine exchange), manganese (is contained in amino-acyl-t-RNA-synthetases), selenium (is contained in the antioxidant system of cells), in deficiency of the latter elements of an erythron are damaged by active oxygen radicals, and the life span of erythrocytes reduces, etc.

Nervous regulation of hemopoiesis, erythropoiesis in particular, suggested still by S. P. Botkin (1884), is confirmed by the results of experimental and clinical observations. Thus, in experimental neuroses anemia and reticulocytopenia develop. Stimulation of the posterior hypothalamus stimulates, while that of the anterior hypothalamus — inhibits erythropoiesis, after removal of the cerebellum a megalocytic anemia may develop. Anemia develops also in denervation of the sinocarotid reflex zone, the spleen, kidneys, small intestines etc.

Erythropoiesis is also regulated by the endocrine system. It is established in experiments on animals, that hypophysectomy causes the development of megalocytic anemia, reticulocytopenia; hyperfunction of the hypophysis is followed by polycythemia. ACTH (adrenocorticotrophic hormone) — increases the erythrocyte and hemoglobin content in the peripheral blood; somatotropine potentiates the reaction of erythropoietine-sensitive cells to erythropoietine; adrenal hormones possess the ability of stimulating erythropoiesis; male sex hormones stimulate, while female inhibit erythropoiesis, that partially explains the difference in erythrocyte count of males and females.

### 3.3. PATHOLOGIC CHANGES OF ERYTHROCYTES

Changes of erythrocytes may be quantitative (decrease, increase of the number) and qualitative (change of size, shape, coloration, appearance of impurities).

There are regenerative forms of erythrocytes, their appearance in the peripheral blood evidences a good or increased hemopoietic function of the bone marrow; and degenerative ones that manifest perverted, impaired hemopoiesis.

**Regenerative forms of erythrocytes** appear in the peripheral blood after an acute blood loss, in acute hemolytic crisis, successful treatment of a number of anemias. Potentiation of regenerative processes is manifested by:

– the appearance of nuclear progenitors of erythrocytes — normoblasts (normocytes), polychromatophilic and oxiphilic;



– an increase of the number of polychromatophiles — polychromatophilia;

– an increase of the reticulocyte count (in norm — 0.2–1.0 %) — reticulocytosis. Granulo-reticulo-filamentous substance is revealed in supravital staining and in those erythrocytes, which in staining on Romanovsky-Gimsa seem to be completely homogenous. Thus, the supravital staining reveals latent basophilia of the cytoplasm.

The number of reticulocytes in the peripheral blood is the main indicator of the functional state of erythropoiesis, regenerator possibilities of an erythron, as increased entering of reticulocytes from the bone marrow is usually combined with enhancement of physiologic regeneration of erythrocytes. However, sometimes an enhanced peripheral reticulocytosis is not an indicator of increased erythropoiesis, but of an increased erythropoiesis — diapedesis of erythrocytes from the bone marrow into the circulating blood (e. g., in irritation of the bone marrow by cancer metastases): that is why, evaluating peripheral reticulocytosis one should bear in mind, that it has a positive meaning only in case, when it is transient and precedes an increase of the erythrocyte count. Reticulocytosis that persists for a long time and is not accompanied by an increase the erythrocyte count, does not exclude a hypoplastic condition of the bone marrow.

Degenerative forms of erythrocytes are presented in table 1.

Table 1

**Degenerative forms of erythrocytes**

Name and description of a cell	Clinical manifestation
1. Change of sizes (anisocytosis)	
Microcyte. MCV decreased. Hypochromia	In iron-deficient anemias and thalassemia
Macrocyte (round or oval shape). MCV increased. A pale area in the center is slightly marked	In megaloblastic anemias, in alcoholic lesions of the liver, after splenectomy
Megalocyte. MCV) increased (12–15 µm in diameter), sometimes of irregular shape, MCH increased ( <i>HbF</i> ), intensely stained	In megaloblastic anemias
Anisocytosis is revealed practically in all types of anemia, its degree corresponds to the severity of anemia	
2. Change of the shape (poikilocytosis, often combines with anisocytosis)	
Poikilocytes. Cells of odd shape — elongated, pearshaped, spheric, etc.	In megaloblastic, iron-deficient anemias, thalassemia, burns, etc.
Spherocyte, can be micro-, normo-, macrocytic. A pale area in the center is absent. More often a macrocyte with increased MCHC) and decreased ( <i>MCV</i> )	In hereditary spherocytosis and other hemolytic anemias, when the erythrocyte membrane is removed in the spleen or RES), but the amount of hemoglobin remains constant
Echinocyte — a toothlike cell, reminding a sea-urchin in shape	In uremia, gastric cancer, peptic ulcer complicated by bleeding, blood transfusion containing old erythrocytes, sometimes — artifact

<b>Name and description of a cell</b>	<b>Clinical manifestation</b>
Acanthocyte — a leaf-like, spur-like cell. Has protrusions of various degree, located on the cell surface at various distances from each other	In alcoholic lesion of the liver, hyposplenia
Degmacyte («nibbled» cell). The erythrocyte looks like it is nibbled	In deficiency of G-6-PD; hemoglobin instability, in removal of Heinz bodies with a part of the membrane and hemoglobin in RES
Schistocyte (helmet-like cell, fragmented cell)	In hemolytic anemias of any etiology with intravascular hemolysis
Drepanocyte — a sickle-cell	In sickle-cellular anemia
Cameloid (Ovalocyte, eliptocyte). A cell of oval or elongated shape. Pallor in the center is not visible. Abnormalities of hemoglobin or membrane cause a change of the cell shape	In hereditary eliptocytosis (ovalocytosis), talassemia, megaloblastic anemia, iron deficiency
Codocyte (torocyte) — target-like erythrocyte, a bell-like cell. If to look at the cell from the side, it resembles two adjoined Mexican hats	In talassemia, iron deficiency, after removal of the spleen, hepatic diseases. Osmotic resistance of cells is increased due to thickening of the membrane
Stomatocyte (mouth-like cell) — a cup-like erythrocyte	In hereditary spherocytosis and stomatocytosis, alcoholism, liver pathology, under the action of medicines
Dacriocyte (spleen-like cell, reminds a drop or a tadpole)	In myelofibrosis, talassemia, anemia in myelophthisis, myeloid metaplasia
Vesicular cell. Looks as if it has a vesicle or blister on its surface	In immune hemolytic anemia. The mechanism of its formation is not clear
<b>3. Intracellular inclusions in erythrocytes</b>	
Jolly's bodies (Hawell-Jolly). The remainder of the nucleus as 1–2–3 basophilic lumps	In the absence of the spleen, intensive hemolysis, megaloblast anemia, lead intoxication; the result of damaging nucleus involution
Kabo's rings (Kabbot). The remainder of the nuclear membrane as a ring, figure of 8, are formed of mitotic filaments or nuclear membrane	In megaloblastic, hemolytic anemias, lead intoxication; the result of damaging the nucleus involution
Basophil granularity (puncture). Disseminated granules of a blue color, revealed in staining according to Romanovsky-Gimza	In lead and other intoxications, sideroblast and megaloblast anemias, talassemia; the remains of basophilic substance of the cytoplasm — the result of damaging its involution
Heinz's bodies. Blue-rounded, single or multiple inclusions, formed of denaturated hemoglobin. Are revealed in supravital staining by crystal-violet-acetyl-phenyl hydrazine	In insufficiency of G-6-PD erythrocyte, action of hemolytic poisons

Name and description of a cell	Clinical manifestation
Pappenheim's bodies (siderous granules) — dark-blue granules of 3-atom iron. Containing their erythrocytes — siderocytes. Increasing of siderous granules — a sign of overflowing of the organism with iron or its inability to utilize it. The absence — a sign of iron deficiency	Increasing — in sideroblast, hemolytic anemias, hypersplenism; absence — in iron deficient anemia
4. Change of coloration	
Hypochromia — slightly stained erythrocytes, have a shape of a ring (anulocytes). Decreasing of MCH	The result of non-saturation of normal in volume erythrocytes with hemoglobin, or microcytosis (false hypochromia). The indicator of iron deficiency in the organism or its dissimulation by erythrocytes in the impairment of hema synthesis. In all iron-deficient and iron non-saturated anemias (sideroblastic, sideroachrestic) anemias
Hyperchromia — intensely stained erythrocytes. Increasing of <i>MCH</i> depends on increasing of <i>MCV</i> , but not on increased saturation of erythrocytes with hemoglobin. Is always combined with macromegalocytosis	In megaloblast and macrocyte anemias

Hb saturation degree of erythrocytes is determined by the color factor (CF), having an important diagnostic significance for revealing normo-, hyper- and hypochromia.

If the blood contains 160 g/l of *Hb* and  $5.0 \cdot 10^{12}/l$  of erythrocytes, then CF is equal to 1.0. In norm CF is equal to 0.8–1.0. CF is calculated as follows.

$$CF = \frac{Hb \text{ g/l}}{160 \text{ g/l}} \cdot \frac{E/l}{5,0 \cdot 10^{12}/l}, \text{ if } E = \alpha \cdot 10^{12}/l,$$

$$\text{then } CF = \frac{Hb \text{ g/l} \cdot 5,0 \cdot 10^{12}/l}{160 \text{ g/l} \cdot \alpha \cdot 10^{12}/l} = \frac{5,0 \cdot Hb}{160 \cdot \alpha} = \frac{5 \cdot Hb}{1,6\alpha \cdot 10^2} = \frac{3Hb}{\alpha \cdot 10^2}$$

Degenerative forms of erythrocytes also include the cells of a megaloblast type of hemopoiesis.

### 3.4. TYPICAL FORMS OF IMPAIRMENTS AND REACTIVE CHANGES IN THE SYSTEM OF ERYTHROCYTES

There are two main groups of typical forms of impairments and reactive changes in the system of erythrocytes: anemias and erythrocytoses.

#### 3.4.1. Anemias. General characteristic. Classification

**Anemia** — is the condition characterized by a decrease of the total volume of an erythrone accompanied by a decrease of the erythrocyte count in a blood volume unit and (or) *Hb*, sometimes associated with their qualitative

changes. In the majority of cases it is a syndrome developing in various diseases, sometimes being a main, central manifestation of the disease.

There are many **classifications of anemia**, based on various principles. Classification by a pathogenetic principle is widely used (table 2).

Table 2

**Types of anemia**

Criteria	Anemia	Notes
Etiology	1. Primary (hereditary, congenital) 2. Secondary (acquired)	
Pathogenesis	1. Posthemorrhagic caused by blood loss 2. Diserythropoietic (caused by the impairment of hemopoiesis) 3. Hemolytic (caused by intensification of blood destruction)	
Type of hemopoiesis	1. Erythroblastic (normoblastic, normocytic) 2. Megaloblastic (megalocytic)	
Color factor	1. Normochromous 2. Hyperchromous 3. Hypochromous	0.85–1.05 >1.05 <0.85
Mean volume of erythrocytes ( <i>MCV</i> )	1. Normocyte 2. Microcyte 3. Macrocyte 4. Megalocytic	80–100 pfl ( $10^{-15}/l$ ) <80 pfl >80–100 pfl >120–150pfl
Velocity of development and duration	1. Acute 2. Chronic	Develop within some days; Last for some weeks, years
Regenerative ability of an erythrocyte hemopoietic germ (on the reticulocyte index; $N = 0.2-1\%$ )	1. Regenerator, hyperregenerator 2. Hyporegenerator, aregenerator (aplastic)	>1 %, polychromatophilic and oxiphilic normoblasts <0.2–0 %
Severity degree on Hb	Light, of a moderate severity, severe	

### 3.4.2. Etiology and pathogenesis of some forms of anemia

#### 3.4.2.1. Posthemorrhagic anemias

**Acute posthemorrhagic anemia.** It occurs due to an acute massive blood loss (see. part 1.1. «Blood loss»). The peripheral blood changes have a phasal character. On the 2<sup>nd</sup>–3<sup>rd</sup> day after the blood loss the amount of erythrocytes and hemoglobin decreases, the hematocrit falls down, but due to the exit of erythrocytes from the depot the color factor is preserved in the norm (normochromous anemia); there develops leucopenia (loss of leukocytes in blood loss, hemodilution), thrombocytopenia (loss of thrombocytes in blood loss, consumption in thrombus formation). Moderate anizocytosis and poikilocytosis of erythrocytes is observed. The resulting hypoxia leads to an increase of

the erythropoietine level and on the 4<sup>th</sup>–5<sup>th</sup> day after hemorrhage the function of the bone marrow is activated (a medullary phase of compensation), regenerative forms of erythrocytes appear — polychromatophiles, single normoblasts (polychromatophile, oxiphilic), reticulocytosis. Anemia acquires a hypochromous character, as accelerated regeneration passes ahead of erythrocyte maturation due to iron deficiency. Neutrophile leucosis develops with a shift to the left on a regenerative type.

The development causes of IDA (iron-deficient anemia) may be: 1) chronic, even not profuse and latent blood losses.

**Chronic posthemorrhagic anemia.** It develops due to prolonged recurrent small hemorrhages. It is a variant of iron-deficient anemia, its pathogenesis and manifestations are associated with growing iron deficiency.

### **3.4.2.2. Diserythropoietic anemias (due to the impairment of erythropoiesis)**

This group of diseases includes:

a) anemias associated with the impairment of cessation of erythropoiesis as a result of deficiency of substances necessary for normal hemopoiesis — deficient anemias ( $B_{12}$ -,  $B_6$ -,  $B_2$ -folic-deficient, iron-copper-cobalt-deficient, protein-deficient), as well as anemias occurring in inability of the bone marrow to assimilate hemopoietic factors — achrestic (siderachrestic,  $B_{12}$ -achrestic, etc.);

б) anemias caused by the damaging the bone marrow by toxic and medicinal substances, ionizing radiation (aplastic);

в) anemias in leukemias, metastases of tumors into the bone marrow (metaplastic).

**Iron-deficient anemia (IDA).** Iron is one of the most important microelements contained in the human organism. An adult needs 15–18 mg/day of iron, of which 2–2.5 mg are absorbed. The biological availability of the hemoous iron contained in food is substantially higher than that of non-hemoous iron. The basic depot of iron is the liver (hepatocytes and macrophages), bone marrow, spleen, muscles. If the iron metabolism is normal, 30–40 % of normoblasts of bone marrow contain granules of ferritine (sideroblasts). The absence of sideroblasts is characteristic of iron deficiency. The excess of granules in a sideroblast is a sign of overflowing the organism with iron (hemociderosis) or inability of its utilization (sideroblast anemia, see below).

IDA comprises about 80–90 % of all cases of anemia and is one of its most common forms. Women suffer more often than men, as iron stores in the latter are considerably higher (by 100–200 %) than those in women. Obvious and latent iron deficiency is noted in almost in 60 % of women in the world.

The development causes of IDA may be: 1) chronic, even unstable and latent blood losses; 2) insufficiency of taking iron with food; in economically developed countries it occurs rare in adult persons, more often — in developing countries, in children — in artificial feeding with cow or goat milk; in neonates

it is the result of iron deficiency in mothers during pregnancy, in premature newborns, in polyploidy; 3) intense consumption of iron during growth and maturation, during pregnancy, lactation; 4) decreased absorption of iron after resection of the stomach, a part of the small intestine, intestinal diseases; syndrome of malabsorption; 5) impairment of metabolism and utilization of iron in infections, intoxications, helminthes invasions; the latter especially often occur in countries with hot climate (ankilostomidosis, schistosomiasis, etc.); 6) large chronic foci of infection, fast growing tumors (redistribution deficiency of iron); in erythremia a considerable tissue deficiency of iron may develop due to frequent therapeutic blood lettings and accumulation of iron in hemoglobin of a neoplastically enlarged erythron; 7) impairment of iron transport (hypo-, atransferrinemia). Frequently combinations of mentioned factors come into action.

The basic sign of the disease is a decrease of the hematocrit. The content of *Hb* fluctuates depending on the severity of anemia from 30 to 100–110 g/l. The erythrocyte count is decreased to a lesser degree than the level of *Hb* (but it may be even normal). CF is sharply reduced (0.6–0.3), hypochromia, microcytosis, anizo- and poikilocytosis of erythrocytes (fig. 4). Often neutropenia develops (as a result of reduction of iron-bearing enzymes in leukocytes). ESR is slightly increased. The content of iron in the serum is decreased (sideropenia) — up to 2.0–5.0  $\mu\text{M/l}$  (in norm — 12–32  $\mu\text{M/l}$ ). The level of thrombocytes may be slightly elevated (on the background of hemorrhages). The reticulocyte index more often corresponds to a hyporegenerative condition.

**Clinical picture** is composed of 2 main syndromes: general anemic and sideropenic.

*The general anemic syndrome* is manifested by symptoms characteristic of all forms anemia: pallor, general weakness, early fatigue, fainting, breathlessness, tachycardia, systolic murmur.

*The sideropenic syndrome* is characterized by a number of trophic impairments. There is marked: dryness and fissures of the skin, premature wrinkles, fragility of nails, koilonichia (katlonichia) — spoon-like nails, angular stomatitis, atrophy of mucous membranes of the mouth, epigastrium, stomach and respiratory ways. The immunity is impaired leading to chronization of infections, frequent ARD (acute respiratory diseases; muscular weakness and weakness of physiologic sphincters develops. There may occur perversion of taste (eating of uneatable products — chalk, paper, etc.), craving for unusual smells (acetone, benzene, paint). The memory and attention concentration are impaired. Sometimes «sideropenic subfebrilitet» occurs.

**Iron-saturated** (sideroachrestic, sideroblastic, iron-refractory) **anemia** includes a group of hereditary or acquired anemia, when the activity of enzymes participating in synthesis of porphyrins and hem is impaired.

**Hereditary forms** are more often presented by anemia, transmitted by a recessive gene, localized in the X-chromosome; rarely this anemia is trans-

mitted by recessive-autosomal inheritance. The genetically determined impairment of enzymes and co-enzymes activity that participate in hem synthesis leads to a decrease of the amount of formed protoporphyrines and activity of the iron binding process. The latter is accumulated in the organism and is deposited in organs conditioning an appropriate clinical picture (when iron is deposited predominantly in the liver, liver cirrhosis develops, and if in the cardiac muscle — circulation insufficiency, etc.).

The development of anemia due to a gene localized in the X-chromosome is associated with a defect of piridoxalphosphatase (pyridoxine-dependent). It is confirmed by a favorable therapeutic effect of piridoxalphosphate and vitamin  $B_6$ .

Anemias caused by the impairment of other enzyme systems are pyridoxine-resistant.

**Acquired forms** develop in using anti-tuberculosis preparations possessing antagonistic action to piridoxine; in deficiency of vitamin  $B_6$ , chronic alcoholism, in lead poisoning (saturnism) as a result of lead blocking of sulphohydroxilic groups of enzymes participating in the synthesis of hem, in chronic diseases.

The erythrocyte count decreases to a lesser degree than the content of *Hb*. CF reaches 0.6–0.4. There takes place marked hypochromia, basophilic punctuation of the cytoplasm (sometimes codocytosis), anizocytosis, poikilocytosis of erythrocytes. The content of iron in the blood serum is considerably increased (60–90  $\mu\text{mol/l}$ ). The content of leukocytes, thrombocytes, the leukocyte formula are normal, if the hepatic function is not impaired. The amount of sideroblasts in the bone marrow increases.

The clinical picture in lead poisoning is characterized by the impairment of the nervous system (encephalopathy, polyneuritis and paresis) and gastric intestinal tract (decrease of appetite, «lead» colic, a violet fringe on the gums — a consequence of lead deposit in cells).

#### ***Anemias associated with deficiency of vitamin $B_{12}$ and folic acid***

**$B_{12}$ -deficient anemia.** Its classic variety — anemia in Addison-Birmer's disease (malignant, pernicious), is manifested by a triad of symptoms: 1) the impairment of hemopoiesis; 2) atrophic changes of the mucus of the gastric intestinal tract; 3) impairments on the part of the nervous system.

**Etiology.** Exogenous deficiency of vitamin  $B_{12}$  occurs rarely. Endogenous deficiency may occur in decrease or complete suppression of gastromucoprotein production by parietal cells of the stomach caused by: a) a hereditary defect transmitted autosomally-recessively (is revealed in  $\frac{1}{3}$  of patients); b) immune mechanisms (antibodies to internal anti-anemic factor or parietal cells of the stomach are revealed in 50 % of patients); c) toxic action on the mucus of the stomach; d) gastroectomy; e) gastric cancer, etc. Endogenous insufficiency occurs in the impairment of absorption of vitamin  $B_{12}$  in the intes-

tines (resection of a small intestine, enteropathy, etc.), in increased consumption of vitamin B<sub>12</sub> (pregnancy, invasion of wide lentetsa).

**Pathogenesis.** In norm vitamin B<sub>12</sub> (external anti-anemic factor) forms a complex with gastromucoprotein (internal anti-anemic factor), which interacts with specific receptors in the inferior and middle part of the ileum, providing the absorption of vitamin B<sub>12</sub>. About 1 % of vitamin B<sub>12</sub> may be absorbed independently of the internal factor. One of co-enzymes of vitamin B<sub>12</sub> — methylcobalamine participates in normal hemopoiesis. With its participation from uridinemonophosphate, timidinemonophosphate is formed, it being a component of DNA. For the synthesis of timidinemonophosphate one needs also folic acid. In the absence of methylcobalamine no DNA is formed, the division processes of actively regenerating cells are impaired, erythropoiesis reacts to them most sharply; a normoblastic type of hemopoiesis passes into a megaloblastic one. The latter is characterized by a smaller number of mitoses (instead of three mitoses characteristic of normoblastic erythropoiesis, only one mitosis occurs), prolongation of the mitotic cycle, early hemoglobinization of megaloblasts,

decrease of osmotic resistance of megalocytes, reduction of their life span, increase of ineffective erythropoiesis, decrease of erythrocytes life span. Extramedullar foci of megaloblastic hgemopoiesis appear. Leuko- and thrombocytopoiesis are also impaired. The second co-enzyme — desoxiadensilcobalomine takes part in exchange of fatty acids, in transformation of a methylmalonic acid into a succinic one. In deficiency of vitamin B<sub>12</sub> methylmalonic acid is accumulated in the organism, it causes dystrophy of posterior-lateral columns of the spinal cord, the development of funicular myelosis, functional impairment of the central nervous system.

The blood pattern is characterized by a sharply marked hyperchromous anemia (CF > 1.0). The erythrocyte count decreases to a greater degree than *Hb*, leucopenia with neutropenia, relative lymphocytosis, thropmbocytopenia. The smear reveals megaloblasts, megalocytes, anizocytosis, poikilocytosis, macrocytosis, erythrocytes with Jolly's bodies, Kabo's rings, basophilic granularity, giant polysegmentnuclear neutrophiles, the count of reticulocytes decreases (its increase evidences the remission), ESR increases. In the bone marrow, sometimes oxiphilic megaloblasts are absent, basophilic forms prevailing («blue bone marrow»). Degenerative changes are noted in cells.

The impairment in the gastric intestinal tract and nervous system aggravate the course of anemia. Glossitis of Gunter (inflammation with subsequent formation of a «varnished» tongue due to atrophy of its papilla), stomatitis, gastroenterocolitis develop. The neurologic syndrome is manifested by psychic disturbances (delirium, hallucinations), swaying gait, paresthesia, pain sensations, numbness of extremities, parapareses, occurrence of pathologic reflexes, etc.



**Folic-deficiency anemia.** The development cause of this disease is insufficient consumption of folic acid by the organism with food (fasting, especially in childhood in feeding only with goat milk; it occurring often in hot countries); the impairment of absorption (intestinal malabsorption, alcoholism, enteritis, enteropathy, administration of some medicines); increased need in folic acid and its consumption (pregnancy, lactation).

The insufficiency of folic acid in the organism causes the impairment of synthesis and DNA structure causing the transition of a normoblast type of hemopoiesis to a megaloblast one with all associated consequences. The blood picture and clinical manifestations of this disease are similar to those of B<sub>12</sub>-deficient anemia, however a gastroenterocolitic and neurologic syndromes being absent.

**B<sub>12</sub>-achrestic anemia.** In this anemia the process of producing the internal anti-anemic factor is not impaired and changes on the part of the digestive and nervous system are absent. The development of this anemia is associated with the impairment of metabolism of methylcobalamine; as a result the bone marrow loses its ability of utilizing hemopoietic substances and megaloblast erythropoiesis occurs. The blood picture is the same as in B<sub>12</sub> and folic-deficient anemia. The content of vitamin B<sub>12</sub> in the blood plasma may be normal or elevated.

***Aplastic anemias. The syndrome of bone marrow insufficiency***

Anemias of this group may be acquired (secondary) and hereditary, congenital (primary).

**Acquired forms** may develop under the effect of physical (ionizing radiation); chemical (benzole, arsenic, etc.) factors, medicinal preparations (some antibiotics — levomecycytine, sulphonilamides, etc.) and also due to insufficiency of hormones (myxedema, hypophyseal insufficiency); occurrence of malignant tumors; viral infections; action of autoantibodies.

In this disease stem cells and precursor cells of myelopoiesis are predominantly damaged.

**Hereditary aplastic (constitutional anemia of Fancony) is transmitted on autosomal-recessive type.** The pathology of hemopoietic cells is caused by a defect of  $\gamma$ -endonuclease enzyme taking part in the work of the reparse antimutation system of cellular nuclei.

Due to this the repair processes of stem cells DNA with increased mutability are impaired, which is evidenced by high incidence of leukemia in patients with Fankony's anemia.

Aplastic anemias are the main manifestation of the **syndrome of medullary insufficiency**. This condition is characterized by: a decrease of the hemopoietic tissue volume; substitution of bone marrow for the adipose tissue; pancytopenia in the peripheral blood (expressed anemia, *Hb*-20–30 g/l, normochromia, microcytosis, decreased count of reticulocytes, increased content of

*HbF*, leukopenia, absolute neutropenia, relative lymphocytosis, thrombocytopenia, elevated ESR); general anemic syndrome (pallor, listlessness, breathlessness, etc.); immune-deficiency syndrome (infections, sepsis); hemorrhagic syndrome (petechiae, bruises, hemorrhages); hemolytic syndrome (short-lived erythrocytes); increase of the iron content in the blood serum caused by the impairment of iron inclusions into hemoglobin (saturation of transferrin with it reaches 100 %); high level of erythropoietine in blood in decreased efficiency of its effect on the bone marrow.

**Metaplastic anemia.** This pathology occurs in overgrowing of cells in bone marrow that have nothing to do with erythropoiesis (acute leukemias, multiple myeloma, myelofibrosis, osteomyelosclerosis, metastases of tumors). The blood picture is determined by the primary disease.

### 3.4.2.3. Hemolytic anemias (HA)

#### *Hereditarily conditioned hemolytic anemias (primary)*

**Erythrocytopathies.** The most common are — *hereditary family spherocytosis* (microspherocytosis, disease of Minkovsky-Shoffar, protein-dependent membranopathy). The disease is inherited by an autosomal-dominant way. In its base is the defect of erythrocyte membrane structure causing a change of their shape from discoid to a spherical one. Such erythrocytes are not deformed and when passing through narrow capillaries they lose a part of membrane substance, diminish in size and are destroyed. Their membrane becomes highly permeable for ions of Na and water. In the blood with sufficient glucose the pump of Na provides the excretion of Na excess. In intrasinusoidal spaces of the spleen, where the glucose content is decreased, Na is not excreted resulting in osmotic hemolysis of erythrocytes. The basic clinical manifestations of the disease are periodic hemolytic crises, anemia, jaundice, splenomegaly, urobilinemia, urobilinuria, elevation of temperature, trophic ulcers of leg as a result of microthrombosis. Meanwhile the content of *Hb* and erythrocytes in blood decreases, there develops normochromia, microspherocytosis, reticulocytosis (10 % and over), osmotic resistance of erythrocytes decreases. During hemolytic crises neutrophilic leukocytosis is observed.

Hereditarily conditioned erythrocytopathies (membranopathies) also include *ovalocytosis (eliptocytosis)*, *stomatocytosis*, *akanthocytosis* and other HA that gained their name due to a characteristic shape of erythrocytes.

**Fermentopathies (enzymopathies) include a group of HA** that are manifested by insufficient activity of erythrocyte enzymes participating in the process of their energetic supply. In countries of the Mediterranean Sea, Latin America, Africa, Asia often occurs *anemia caused by deficiency of activity of glucose-6-phosphatedehydrogenase (G-6-PDH)* of erythrocytes. There are two basic mutant forms of this enzyme. One of them (form *B*) is common among Europeans, the other (form *A*) — among the black population of Africa. The disease is transmitted on a codominant type, is linked with X-chromosome

and is clinically manifested mainly in males. In females the expressed clinical picture is possible only in case they are homozygous on the given gene.

In insufficient activity of G-6-PDH in erythrocytes, aerobic oxidation of glucose is impaired, which weakens the formation processes of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and restoration of glutathione necessary for protection of *Hb* and erythrocyte membrane from oxidants including medicinal substances. In taking usual therapeutic doses of medicines — oxidants (anti-malaria preparations, sulphanilamides, derivatives of salicylic acid, etc.) there occurs oxidation of *Hb*, hemoglobin disappears from its molecule, hemoglobin chains sediment as Heinz bodies. Erythrocytes are released from them in the spleen. Due to this a part of their membrane substance is lost, they undergo hemolysis, a hemolytic crisis develops, which stops only when all erythrocytes with G-6-PDH deficiency are destroyed (the phenomenon of «self-restriction» hemolysis). A similar picture is observed in taking of horse beans with food (favism — «Baghdad spring fever», is common in Iraq, when leguminous plants are in bloom), sometimes in viral infections, hypovitaminoses of P, C, E, poisonings with aniline, benzole, phenylhydrazine, as a result of taking great amounts of blueberries, bilberries, inhaling the pollen of grass, trees, etc. (the disease occurs in Belarus).

Hemolytic crises are characterized by: high temperature, headache, adynamia, hemoglobinuria, jaundice, hepatomegaly. These events are caused by released inflammatory mediators in damage of erythrocytes, including pyrogenic cytokines.

The blood picture reveals: anemia in a severe degree, reticulocytosis, erythrocytes with Heinz's bodies, anisocytosis, poikilocytosis, degmacytes, schisocytes, basophilic punctuation of erythrocytes, normoblastosis, neutrophilic leukocytosis with a shift to the left (to myelocytes).

**Hemoglobinopathies (hemoglobinoses)** occur as a result of hereditary impairments of globin synthesis. They may be qualitative due to changes of the primary structure of *Hb* (sickle-cell anemia), and quantitative due to the impairment of the synthesis velocity of one of globin chains (thalassemia). The majority of hemoglobinopathies is inherited autosomally-dominantly. This pathology occurs mainly in countries with hot climate: in Central Africa, Asia and Cuba. Homozygous pathogenicity gives high children's mortality.

**Sickle-cell anemia (hemoglobinopathy S, drepanocytosis)** — is the most common form of pathology associated with abnormality of *Hb* structure. It is spread in many tropical regions of Africa where malaria has an endemic character. This pathology occurs, when in  $\beta$ -chain of *Hb* the glutamine acid is substituted for valine that changes physical-chemical properties of a hemoglobin molecule (*HbS*). In the altered condition the solubility of *HbS* is sharply decreased, the molecules aggregate resulting in the formation of jelly and crystals. The appearing polymers present long filaments grouped in so-called tactoids.

The latter change the shape of erythrocytes, and sickle-shaped erythrocytes (drepanocytes) appear, which are easily exposed to hemolysis.

Clinically the disease manifests in case, when the content of *HbS* in erythrocytes exceeds 45 % or less, when the patient gets under the conditions of decreased partial pressure of oxygen (high mountains, a flight at a high altitude, etc.). It is accompanied by periodical hemolytic, aplastic, polyuretic, nocturic, acute pain, occlusive sequestration crises. They are provoked by hypoxia and acidosis of any origin. Pain attacks are associated with aggregation of drepanocytes in the blood flow, formation of microemboli, vascular microthrombosis with development of infarctions of various organs, strokes, «breast syndrome» (occlusion of the pulmonary artery branches), ischemia and exfoliation of the retina. The sequestration crisis is due to a sudden accumulation of blood in the spleen, less frequently in the liver. Due to occlusion of vessels this blood is quickly isolated from the blood flow. This threatening to life complication develops more often in children of younger age. There develops hypovolemic shock, hepato- or splenomegaly. Chronic hypoxia and impairment of blood viscosity result in hyperfunction of the myocardium and overloading cardiac insufficiency. The secondary immunodeficiency is noted in the patients.

The blood pattern of this disease is characterized by anemia with a considerable decrease of erythrocytes and *Hb*, hypo- or normochromia, anisocytosis, poikilocytosis, basophilic punctuation of erythrocytes, the presence of drepanocytes, reticulocytosis, sometimes normoblastosis, during the hemolytic crisis — neutrophilic leukocytosis with a shift to the left, thrombocytosis.

**Talassemias (disease of Cooley, Mediterranean anemia)** unite a group of hereditary anemias, when the presence of a mutation gene results in synthesis inhibition of hemoglobin chains, deficiency of *HbA*.

There is  $\alpha$ - и  $\beta$ -thalassemia. More often occurs  $\beta$ -thalassemia, when synthesis of  $\beta$ -chains of globin is absent or decreased. In this case the amount of *HbA* decreases, it contains per two  $\alpha$ - and  $\beta$ -chains, while the content of *HbA<sub>2</sub>* (per two  $\alpha$ - and  $\delta$ -chains) и *HbF* (per two  $\alpha$ - and  $A\gamma$ -chains) increases. Excessively synthesized  $\alpha$ -chains form unstable *Hb*, there occur its precipitates, bearing those erythrocytes are removed by the cells of the macrophageal-phagocytic system. It is accompanied by damaging of the erythrocytes membrane; excessive  $\alpha$ -chains interacting with SH-groups of this membrane increase its permeability, which also contributes to activation of hemolysis of erythrocytes. Hem synthesis and iron metabolism are impaired.

Homozygotes develop a severe hemolytic anemia (great talassemia, Cooley's disease), resulting in high children's mortality on the 1<sup>st</sup> or 5<sup>th</sup>–8<sup>th</sup> year of life.

In the impairment of synthesis of  $\alpha$ -chains occurs  $\alpha$ -thalassemia. Homozygous pathogenicity results in intrauterine death of the fetus, heterozygous — in hemolytic anemia of various severity. The blood picture reveals hypochromous

anemia (CF = 0.5–0.4), anisocytosis, microcytosis, poikilocytosis, hypochromia, a great number of target-like erythrocytes (torocytes), basophilic punctuation of erythrocytes; reticulocytosis (5–10 %), moderate neutrophilic leukocytosis with a shift to the left, a level increase of the serum iron.

The spread of hemoglobinoses coincides with so-called malaria zones of the Earth. It turned out that carriers of *HbS* and those ill with thalassemia either don't fall ill with tropic malaria or suffer it in a light degree. The resistance of patients with hemoglobinoses to malaria is explained by the fact that their causative agents are intracellular (intra-erythrocyte) parasites. They consume a great amount of oxygen provoking hemolysis of erythrocytes, in the process of which they die themselves.

**Acquired hemolytic anemias** occur, when autoantibodies to their own erythrocytes appear (autoimmune); under the effect of isoimmune antibodies (transfusion of incompatible blood, hemolytic disease of neonates); medicines (sulphanilamides, etc.) mechanic damage of erythrocytes (implanting of heart valves, march hemoglobinuria, etc.); viral infections, exposure to chemical and physical factors (salts of lead, venoms, burns, ultraviolet radiation, etc.). Hemolysis of erythrocytes in this form of anemia is caused by metabolic and structural lesions of their membranes accompanied by further increase of osmolarity of the intracellular content, decrease of the ability of erythrocytes for deformation in sinuses of the spleen, which contributes to their destruction. The blood pattern during the first hours of anemia development reveals short-term «false» hyperchromia, then develops normochromous or hypochromous anemia, normoblastic regenerative or hyperregenerative anemia with polychromatophilia, reticulocytosis, normoblastosis, sometimes with poikilocytosis; leukocytosis. The amount of indirect bilirubin increases in the blood. The syndrome of enhanced hemolysis is combined with the syndrome of enhancement of erythropoiesis. In the bone marrow the number of erythroid germ cells increases.

### 3.4.3. Erythrocytoses

Erythrocytosis is the condition characterized by an increase of the erythrocyte count and *Hb* in a unit of the blood volume, increase of the hematocrit. There are absolute (true) and relative (false) erythrocytoses.

**Absolute erythrocytoses** occur as a result of enhancement of erythropoiesis and are accompanied by an increase of the mass of circulating erythrocytes. They may be primary and secondary.

*Primary erythrocytoses* are independent nosologic forms — diseases. They include erythremia (true polycythemia, Vaquez' disease) — a malignant disease discussed in the group of hemoblastoses (see part 4.5 «Leukemias»).

*Secondary erythrocytoses* are a symptom of this or that disease. More often they develop in hypoxia and enhancement of the erythropoietine production

of (the disease of respiratory organs accompanied by respiratory insufficiency, congenital heart defects, cancer of renal parenchyma, etc.). There may take place moderate polycytemic hypervolemia, an increase of hematocrit, blood viscosity, arterial pressure; there may develop the myocardium hypertrophy, rhythm impairment and contractile function of the heart, skin itching, thrombohemorrhagic syndrome. Besides erythrocytosis, reticulosis is noted in the peripheral blood.

**Relative erythrocytoses** (false) develop due to a decrease of the plasma volume and blood thickening without enhancement of erythropoiesis. The causes of relative erythrocytosis are: dehydration of the organism in profuse perspiration, burns, profuse diarrheas, vomiting.

All listed forms of erythrocytosis are pathologic. **Physiologic** erythrocytosis includes erythrocytosis in residents of high mountains, mountain climbers in the period of acclimatization at high altitudes and after it, as well as in other similar situations of adaptation to chronic hypoxia.

## Chapter 4

### THE SYSTEM OF LEUKOCYTES (LEUKONE) AND ITS IMPAIRMENTS

**Luekone** (white blood) presents a total sum of white blood cells at all development stages as well as the mechanisms of their formation and destruction. The leukone has a myeloid and lymphoid division. The myeloid department consists of a granular and monocytic subdivision.

White blood cells — leukocytes — present a heterogenous population of nuclear-bearing cells, that differ morphologically and functionally; they are divided into granulocytes and agranulocytes. Granulocytes contain cytoplasmatic granules, well seen under the light microscope. By the color of granules, granulocytes are divided into neutropiles, eosinophiles and basophiles.

Agranulocytes are subdivided into lymphocytes and monocytes.

In norm the leukocyte count in the human is  $4-9 \cdot 10^9/l$ . The percentage of some of their types is called a leukocyte formula. The absolute leukocyte count of every type in ablood volume unit is called a leukocyte profile.

#### 4.1. LEUKOPOIESIS

Leukocytes — granulocytes and agranulocytes are formed in bone marrow; lymphocytes become mature basically in lymphoid organs, partially in bone marrow.

##### *Granulocytopoiesis*

*Myeloblast* — is a large cell 12–20  $\mu\text{m}$  in diameter. The cytoplasm of the cell is homogenous, basophilic to a various degree, not granular. The nucleus of the cell is large, round or oval, occupies the most part of the cell, contains delicate, comparatively regularly twisted chromatin and nucleoli (2–6). The cell is identified by a positive reaction to peroxidase and acid phosphatase.

*Promyelocyte* — is a cell larger than a myeloblast, reaches 27  $\mu\text{m}$  in diameter. Differs from a myeloblast by the presence of rough azurophilic granules of a cherry color (primary granules) in the cytoplasm. They serve a source of antibiotic proteins of neutrophilic granulocytes. There is a chrome-bearing enzyme myeloperoxidase in azurophilic granules (the main component of oxidative-cytophagic system of neutrophiles considered as a marker of granulocytes and monocytes) as well as acid phosphatase and other enzymes. Besides azurophilic granules the cytoplasm may contain neutrophilic, basophilic and eosinophilic granules. The nucleus of a promyelocyte is mostly oval, sometimes bean-like and is often located eccentrically. It contains a delicate chromatine net-like structure. The nucleus may have nucleoli.

*Myelocytes* — are cells 10–15  $\mu\text{m}$  in diameter, more often of a round or oval shape. The nucleus of the cell is less than in a promyelocyte and has a more rough structure. Nucleoli are absent. By the character of granularity,

myelocytes are subdivided into neutrophilic, eosinophilic and basophilic. Under normal conditions they are localized only in bone marrow. A neutrophilic myelocyte has specific secondary granules of a pink color or «neutral». They don't contain myeloperoxidase and acid phosphatase, but contain lysozyme and other basic proteins as well as alkaline phosphatase, collagenase, lactoferrin (iron-binding bacteriocidal protein).

*Young leukocyte* (metamyelocyte) — is of a rounded shape, 9–12  $\mu\text{m}$  in diameter. Its cytoplasm occupies the most part of the cell; the nucleus is of a bean-like or sausage shape, rich in chromatin. Metamyelocytes can also be neutro-, eosino- and basophilic.

The nucleus of a *rod-shaped leukocyte* (9–12  $\mu\text{m}$  in diameter) has a shape of a rod or letter S. It is homogenous in thickness, its chromatin is located in a more compact way. Depending on the character of granularity filling in the cytoplasm of the cell, the rod-shaped leukocyte may also be neutro-, eosino- and basophilic.

*Segmentated neutrophile* (9–12  $\mu\text{m}$  in diameter), its nucleus consists of 2–5 segments connected with thin arches. The cytoplasm occupies most of the cell, it is oxiphilic with fine dust-like granularity of a light-pink or violet color.

*Eosinophile* — in the majority of cases it is larger than a neutrophile leukocyte. Its cytoplasm is slightly visible due to light staining and the presence of a great number of granules. The granules are round, rough, of an identical size, refract light well, are stained in orange- or yellow-red color («salmon caviar», «ripe raspberry»). The nucleus of the eosinophile most often consists of two broad rounded segments, rarely of three.

*Basophile* presents some smaller, as compared to a neutrophile, cell (8–10  $\mu\text{m}$  in diameter). The cytoplasm has large granules of various size stained in a dark-violet or dark-blue color. The granularity often may be very plentiful, it covers the nucleus, that is why it is not clearly revealed.

### ***Lymphopoiesis***

*Lymphoblast* — is a cell 15–18  $\mu\text{m}$  in diameter. The cytoplasm is not granulated, basophilic, is stained in a light-blue color. The reaction to peroxidase is negative, to acid phosphatase — sometimes positive. The nucleus is delicate, rounded or oval, contains 1–2 nucleoli.

*Prolymphocyte* — is a large cell 12–15  $\mu\text{m}$  in size. The cytoplasm is basophilic, not granulated, of a blue color («the sky on a clear sunny day»). The nucleus, compared to the nucleus of a lymphoblast, contains a denser chromatin, sometimes nucleoli too. There is a light prenuclear zone around the nucleus.

*Lymphocyte* — is a small cell 8–10  $\mu\text{m}$  in diameter. The cytoplasm is basophilic, surrounds the nucleus with a narrow rim or sickle-like. The nucleus is dense, occupies almost the whole cell, of a very dark color, rounded or bay-like shape. There is a light prenuclear zone around the nucleus.



### ***Monocytopoiesis***

*Monoblast* is a cell 12–16 µm in diameter. Its cytoplasm occupies a comparatively small area, it is basophilic, non granular, is stained in a blue-light, blue or grey-blue color. The nucleus is round, often with an impression, has 1–2 nucleoli. The reaction to peroxidase — is slightly positive; to acid phosphatase — highly positive.

*Monocyte* — is the largest cell of the peripheral blood (12–22 µm in diameter), it is rich in cytoplasm that is stained in a smoke-grey (blue-grey) color («the sky on a clouded day»). There is fine azurophilic granularity in the cytoplasm, is visible in good staining of the smear. The nucleus is large, loose, polymorphous, in a shape of a horse-shoe, bean, trefoil, sometimes as a butterfly with spread wings.

## **4.2. PATHOLOGIC SHAPES OF LEUKOCYTES**

*Neutrophil with hypersegmentation of nuclei.* The presence of more than 5 segments in nuclei of neutrophils is due to the impairment of biosynthesis of nuclear acids there. This pathology occurs in radiation disease or as a result of taking medicines impairing the process of DNA synthesis (hydroxyurea), in giant neutrophils, in deficiency of vitamin B<sub>12</sub> and folic acid («ageing» cells).

*Neutrophile with hyposegmentation of the nucleus* (Pelger-Hewlett's abnormality). Hereditary benign autosomal-dominant impairment of forming nuclei of granulocytes leading to the formation of non-segmentated (in homozygotes) ellipsoid, bean-like, dumb-bell nuclei or bi-segmentated (in heterozygotes) like pince-nez nuclei, when the cytoplasm is normal and mature. In hereditary forms of the disease the leukocyte function stays in norm. Acquired impairment may take place in leukemias, infections, effect of medicinal preparations.

*Leukocyte with toxic granularity.* Rough granularity of a dark color appears in the cytoplasm as a result of protein coagulation in severe infections, intoxications.

## **4.3. TYPICAL FORMS OF IMPAIRMENTS AND REACTIVE CHANGES OF THE LEUKOCYTE SYSTEM**

The typical changes of the leukocyte count in a blood volume unit include leukopenias and leukocytoses. They are not independent diseases but present symptoms of various diseases, pathologic processes, conditions, are of a definite diagnostic importance.

### **4.3.1. Leukopenias**

**Leukopenia** — is the condition characterized by a decrease of the leukocyte count in a blood volume unit less than  $4 \cdot 10^9/l$ .

Leukopenia can be: absolute (decreasing of an absolute count of some types of leukocytes) and relative (a per cent decrease of some types of leukocytes at the expense of an increase of some other their types); physiologic and pathologic. The physiologic leucopenia is the redistribution leucopenia occurring in transferring of a considerable part of leukocytes to some parts of the vascular channel.

*Pathologic leukopenias* can be primary (congenital, hereditary) and secondary (acquired). The primary leukopenias (mainly neutropenias) include leukopenias in syndromes of «lazy» leukocytes and Chediak-Higashi, as well as family neutropenias, chronic granulomatous disease, etc. Secondary leukopenias develop due to the exposure of ionizing radiation, some drugs (sulphanilamides, barbiturates, levomycine, cyclophosphan and other cytostatics) in their prolonged application. They may develop also in immune diseases, autoaggression, generalized infections (enteric fever, paratyphus, the flue, measles, hepatitis), in cachexia, etc.

The following processes underlie the bases of leucopenia pathogenesis:

- impairment and/or depression of leukopoiesis; genetic defect of leucopoietic cells, disturbances of its neurohumoral regulation, deficiency of proteins, vitamin B<sub>12</sub>, folic acid, etc., prolonged administration of medicinal preparations — amidopyrine, etc.;
- excessive destruction of leukocytes in the vascular channel or hemopoietic organs (permeating radiation, antileukocyte antibodies, toxic factors);
- redistribution of leukocytes in the vascular channel (has a transient character); is observed in shock, intense, prolonged muscular work, when a great number of leukocytes migrate into the tissues in their massive destruction (peritonitis, pleuritis, mechanic lesion of soft tissues);
- increased loss of leukocytes by the organism, chronic blood loss, plasmo- and lymphorrhea (extensive burns, chronic purulent processes — osteomyelitis, peritonitis);

In expressed leucopenia the anti-tumor and anti-infection resistance decreases, there is often marked generalization of the septic process, the infection of the organism, neoplasms may develop.

**Agranulocytosis** — is a clinical-hematological syndrome, characterized by decreasing or even disappearing of granulocytes from the blood, leucopenia and appearance of infectious complications («agranulocytic quinsy», stomatitis, necrotic enteropathy, urosepsis, etc.). As agranulocytosis does not clearly differ from granulocytopenia, the clinical course of which has no symptoms, agranulocytosis is assumed to be a condition, when the blood contains less than  $0.75 \cdot 10^9/l$  of granulocytes, and the total leukocyte count is less than  $1.0 \cdot 10^9/l$ . The basic forms of agranulocytosis are: myelotoxic and immune (haptenuous). The causes of *agranulocytosis* are cytostatic preparations, alimentary factors (eating of grains left for winter in the fields) as well as all forms of ionizing

radiation, suppressing precursor-cells of myelopoiesis up to a stem cell, as a result of which not only the count of granulocytes in the blood, but also those of erythrocytes, agranulocytes and thrombocytes decreases. The causes of *immune agranulocytosis* may be unusual sensitivity of the organism to some drugs (sulphanylamides, amidopyrine and its derivatives, barbiturates, etc.). It is accompanied by the formation of anti-leukocyte antibodies. Fixing on the surface of leukocytes they destroy mainly mature granulocytes (sometimes early stages of granulopoiesis too). As a rule, in immune agranulocytosis only the content of leukocytes decreases.

**Panmyelophthisis** (exhaustion of the bone marrow, «consumption» of the bone marrow) — suppression of all functions of the bone marrow: erythro-, leuko- and thrombocytopoietic. It is accompanied by total emptying of the bone marrow — its punctuates reveal only single nuclear elements. The blood reveals growing irreversible aplastic anemia of a hypo-, normo- or hyperchromous character, as well as leucopenia with agranulocytosis and thrombocytopenia.

### 4.3.2. Leukocytoses

**Leukocytosis** — is the condition characterized by an increase of the leukocyte count in a blood volume unit over the norm (over  $9 \cdot 10^9/l$ ). Leukocytosis may be physiologic and pathologic, absolute and relative. *Physiologic leukocytosis* is observed in healthy neonates, in pregnancy, on physical exertion («myogenic»), digestion («digestive»), on psychic stress («emotional»), on changing of time zones («acclimatization»).

*Pathologic leukocytosis* has a different etiology and occurs in various pathologic processes and diseases. They are always secondary to a primary disease and are not permanent.

*Absolute leukocytosis* is revealed by an increase of the absolute number of some types of leukocytes, *relative* — by an increase of their per cent content at the expense of a decrease of other types of leukocytes.

There are some development mechanisms of leukocytosis:

1. Activation of normal leucopoiesis under the effect of leukopoietines (true, absolute leukocytosis). It may be in infections, purulent-septic processes, in aseptic inflammation (allergic reactions, autoimmune diseases, burns, freezing, injuries, myocardial infarction), hemorrhages, poisonings, on radiation.

2. Redistribution of leukocytes in the blood channel (false, relative leukocytosis). It may be observed in traumatic, anaphylactic shock (the leukocyte count increases in the blood of pulmonary microvessels, liver, intestinal walls), on significant physical exertion.

3. Hyperproduction of leukocytes in tumor lesion of the hemopoietic tissue (leukemia).

4. Hemoconcentration. It is caused by hypohydration of the organism, while hypovolemia develops (recurrent vomiting, diarrhea, polyuria).

In leukocytosis not only the total leukocyte count changes, but the leukocyte formula too. According to morphologic signs there may be neutrophile, eosinophile, basophile leukocytosis, lymphocytosis and monocytosis.

#### 4.4. GENERAL CHARACTERISTIC OF SOME TYPES OF LEUKOCYTES, THEIR ROLE IN PATHOLOGIC PROCESSES

The greatest group of leukocytes — is neutrophiles (50–75 %). Their life span is about 15 days.

The basic function of neutrophiles is to protect the organism against infection, everything foreign and that has over lived itself. This process includes chemotaxis, phagocytosis and killing of microorganisms («guards»).

Neutrophiles are the most potent enzyme-formers of all leukocytes. They secrete lyosomal cation proteins and histons into the environment; produce interferon accomplishing an anti-viral function, are the most important macrophages, they clean up the focus of inflammation from microbes and waste products.

For the majority of infections, particularly coccous (strepto- and staphylococcous inflammation — a furuncle, carbuncle, croupous pneumonia, meningitis, acute appendicitis, etc.) are characterized by **neutrophile leukocytosis (neutrophilia)**. It also develops in cancer metastases into the bone marrow, in myocardial infarctions, acute blood loss, etc.

There are the following varieties of neutrophile leukocytosis.

1. Without a nuclear shift — increasing of the number of mature segmented neutrophiles in the blood on the background of general leukocytosis.

2. With a regenerative nuclear shift to the left — on the background of neutrophilia and increased content of rod-nuclear forms, myelocytes (young) are revealed, the normal per cent ratio between the forms being preserved; the total leukocyte count, as a rule, is increased; this form reflects a deeper stimulation of myelopoiesis, the resources for further stimulation being preserved, it occurs in purulent-septic processes. The prognosis is favorable.

3. With a hyperregenerative nuclear shift to the left — is characterized by growing of the number of rod-nuclear neutrophiles, appearance of metamyelocytes, myelocytes, some promyelocytes — an extreme degree of tension of granulopoiesis, an alarming factor evidencing prolonged septic diseases.

4. With a degenerative nuclear shift to the left — increasing of the number of rod-nuclear neutrophiles not accompanied by increasing of young forms; reflects suppression of granulocytopoiesis; is an unfavorable sign, evidences forthcoming functional exhaustion of the bone marrow; the total leukocyte count may correspond to a lower limit of the norm or even moderate leukopenia.

5. With a regenerative-degenerative shift to the left — growing of the number of rod-nuclear granulocytes, metamyelocytes, myelocytes with signs of degeneration (piknosis of nuclei, toxogenic granularity, vacuolization

of the cytoplasm, etc.) is a sign of suppression of medullary functional activity, may take place in severe infectious diseases, endogenic intoxications, etc.

6. A nuclear shift of neutrophiles to the right — the appearance in the blood of a great number of polysegmentated (over 5 segments in the nucleus) neutrophiles on the background of decreasing or disappearing of young cells; reflects the blood picture taking place in primary suppression of granulopoiesis without preceding stimulation; develops in radiation disease, Addison–Birmer disease, scurvy, folic deficiency.

The leukocyte formula shift is characterized by the nuclear shift index (NSI) — the total ratio of all non-segmentated neutrophiles (myelocytes, metamyelocytes, rod-nuclear cells) to the number of segmentated cells (Bobrov's index).

$$\text{NSI} = \frac{\text{Myelocytes} + \text{metamyelocytes} + \text{rod nuclear cells} (\%)}{\text{Segmentated} (\%)}$$

In norm NSI is equal to 0.05–0.1. In regenerative shifts this index increases up to 0.9 and over; in a hyperregenerative shift it increases up to 1–2. In shifts to the right — NSI is less than 0.06.

Decreasing of the absolute neutrophile count in a blood volume unit — is neutropenia. It may be selective (when the number of other leukocytes does not change) or manifest pancytopenia — the deficiency of all corpuscular elements.

Neutropenia is caused by suppression of the neutrophile production process, acceleration of their death; it may be in infections (viral hepatitis, infectious mononucleosis, rubella, the flue, ARVI, HIV-infection, whooping cough, enteric fever, leishminiasis, etc.). In prolonged infections (streptococcos, tuberculosis, meningococcal) «neutropenia of exhaustion» may develop. It is an unfavorable prognostic sign. Neutropenia may also be caused by autoallergy to neutrophile antigens (systemic erythema centrifugum, rheumatoid arthritis, etc.), isoimmune conflict of the mother and child on antigens of neutrophiles, toxic affection of neutrophiles by medicines (sulphonilamides — biseptol, some antibiotics, amidopyrine, aminasine, etc.), as well as poisonings with myelotoxic lyzosomal toxins of a tissue origin (alimentary toxic aleukia) and other factors.

The count of **eosinophiles** in accordance with the leukocyte formula is 2–5 %. The granules of human eosinophiles contain: myeloperoxidase (produces a toxic action on parasites); cation proteins, in particular, eosinophile cation protein, that is the main protein of eosinophiles, produces a parasitocidal action; anti-inflammatory mediators (decrease the intensity of hyperergic inflammation and allergy).

Eosinophiles are capable of chemotaxis and phagocytosis. They reveal their phagocytic activity to immune complexes and parasites, play a protective role in anti-helminthes immunity. Eosinophile peroxidase produces a cytotoxic effect on helminthes.

Increasing of a relative content of eosinophiles (over 5 %) in the blood or their absolute content over  $0.45 \cdot 10^9/l$  — **eosinophilia**. Substantial eosinophile leukocytosis (over 15–20 %, the absolute value is over  $1.5 \cdot 10^9/l$ ) is called essential eosinophilia.

Eosinophilia develops in: allergic processes (bronchial asthma, hay fever, polynoses, atonic dermatitis, angioneurotic edema, urticaria, etc.); parasitic and helminthes diseases (trichinellosis, ascariidosis, echinococcosis, etc.); infections — the period of convalescence — «pink dawn of recovery» (scarlet fever, feverless forms of tuberculosis, tonsillo-genic infection); immune-pathologic diseases (fungal allergic alveolitis, asthma-like syndromes developing in response to penicillin, sulphanilamides and other drugs, bullous eruption, rheumatoid arthritis); immune-deficient conditions; endocrinopathies (primary hypocorticism, Addison's disease, panhypopituitarism); chronic skin diseases (psoriasis, ichthyosis, motley lichen, recurrent granulomatous dermatitis); leukemias and other neoplasms (see below).

The condition when a relative content of eosinophiles decreases in blood (below 2 %) or their absolute count is less than  $0.09 \cdot 10^9/l$  — is **eosinopenia**. The complete absence of eosinophiles is called **eosinophilia**. Eosinopenia and eosinophilia occur in agranulocytosis (on the background of neutropenia), in sepsis, at the beginning of acute infectious diseases. The decrease of the eosine count in progressing leukocytosis evidences the aggravation of the process; aneosinophilia with lymphopenia is an unfavorable sign.

The **basophiles** share is 0–1 % in the leukocyte formula. Like eosinophiles, they perform a desintoxication function. Basophiles are a main source of leukotrienes (leukotriene  $B_4$  is produced only in basophiles). Basophile granules contain peroxidase, heparin, histamine, factor of eosinophile chemotaxis, activation factor of thrombocytes. Heparin of basophiles prevents blood coagulation in the focus of inflammation, while histamine dilates capillaries contributing to resolving and healing.

**Basophilia** can be relative and absolute. Relative basophilia — is increasing of the relative content of basophiles (over 1 % of all white cells) in the blood. Absolute basophilia — is the condition, when the blood reveals over  $0.15 \cdot 10^9/l$  of basophiles. Basophilia occurs in anaphylactic, allergic reactions, in autoimmune diseases (unspecific ulcerative colitis, collagenosis), some helminthoses (ankilostomidosis); a number of autoimmune endocrinopathies (mixedema, thyroiditis, diabetes mellitus of the 1<sup>st</sup> type), myeloproliferative diseases (erythremia, thrombocytopenia, myeloid metaplasia, chronic myeloleukosis), hemophilias, proliferative phase of acute inflammation, viral diseases (chicken pox, grippe), chronic infections (tuberculosis), iron deficiency in the organism, in cancer. It may occur in healthy women too in the period of lactation and at the beginning of periods; also in persons, who suffered splenecto-

my. The absence of basophiles in the peripheral blood (abasophilia) — is a usual phenomenon and not a deviation from the norm.

**Monocytes** after leaving the bone marrow circulate in the blood flow for 20–40 hours and then they migrate to tissues, where their final specialization occurs.

Monocytes that have migrated into the tissues from the blood flow are macrophages (histiocytes of the connective system, Koopfer's hepatic cells, alveolar macrophages, free and fixed macrophages of the spleen, bone marrow and lymphatic nodes, peritoneal macrophages, pleural macrophages, osteoclast, microglial cells of the nervous system).

Monocytes are capable of amoeboid movement and phagocytosis. They phagocytize the remains of their own destroyed cells, malaria plasmodia, various microorganisms and fungi as well as their own ageing cells and those infected with viruses including blood corpuscular elements; clear up the focus of inflammation preparing it for repair («cleaners of the organism»).

An increase of the monocytes count in blood: relative (over 8 %, and for children of early age — over 10 %) or absolute (over  $0.800 \cdot 10^9/l$  — in children,  $0.720 \cdot 10^9/l$  — in adults) — is **monocytosis**. The basic etiological factors of monocytosis are: bacteriemia and infectious inflammation caused by pathogens, that are phagocytized mainly by monocytes (mycobacteriosis, brucellosis, syphilis, enteric fever, glanders, protozoan infections — amoebiasis, leishmaniasis, toxoplasmosis; pox, endemic typhus, measles, malaria); disseminated tuberculosis, leprosy.

Non-infectious causes of monocytosis are: unspecific ulcerative colitis, chronic granulomatous colitis, some forms of autoimmune thyroiditis and immune pathologic cirrhosis of the liver. Monocytosis is accompanied by a number of hemoblastoses (chronic myeloleukosis, lymphogranulomatosis, acute myeloblastic leukemia).

A decrease of the per cent content or absolute count of monocytes — monocytopenias. They may occur in all diseases and syndromes, when the myeloid germ of hemopoiesis is depressed (radiation disease, agranulocytosis, sepsis, etc.).

**Lymphocytes** — are main cells of the immune system. They coordinate and accomplish immune response producing inflammatory cytokines and antigen-specific binding receptors, are responsible for the formation of specific immunity, perform the function of immune surveillance in the organism, provide protection from everything that is foreign, preserving the genetic constancy of the internal environment. Lymphocytes — are non-phagocytizing leukocytes, have no fermentative-receptor apparatus of phagocytosis.

There are *B*-, *T*- и *NK*-lymphocytes. There is an assembled group in the peripheral blood (zero — neither *T*-, nor *B*-lymphocytes), they have no

characters of *T*- and *B*-lymphocytes (immature lymphocytes that are not yet committed into *B*- or *T*-series).

*B*-lymphocytes include antigen-forming cells of a plasmatic series. Having met an antibody, *B*-lymphocytes migrate into the bone marrow, spleen, lymphatic nodes, where they proliferate and transform into plasmatic cells, that are producers of antibodies — immunoglobulins. Their final stages are: a plasmoblast, proplasmocyte and plasmocyte. These cells produce a great number of immunoglobulin molecules of strictly definite specificity. Stimulated *B*-lymphocytes become *B*-cells of long-term memory, they preserve information of a previously met antigen, quickly proliferate and, when they meet a familiar antigen, they produce immunoglobulins. *B*-lymphocytes perform humoral immunity, participate in all kinds of hypersensitivity of an immediate type, in all antibody-mediated forms of immunity (neutralization of toxins and viruses, opsonization in phagocytosis, etc.).

*T*-lymphocytes, are formed of medullary stem cells, are differentiated in the thymus resulting in the formation of mature functionally normal *T*-cells, performing cellular immunity. *T*-lymphocytes perform a number of functions: are carriers of immune memory passing it to *B*-lymphocytes; come into reactions of a cellular type (transplant rejection, «the transplant against the host» reactions, reactions of hypersensitivity of an inhibition type); produce effect on tumors and cells infected by viruses.

There are the following types of *T*-lymphocytes: *T*-killers (cause a reaction of transplant rejection and play a definite role in anti-tumor immunity); *T*-helpers (participate in all immune reactions — humoral and cellular — they produce various cytokines necessary both for humoral and cellular immune response, i. e. they are «helpers» in immune reactions, but they themselves don't produce antibodies); Regulator *T*-lymphocytes (block the process of producing antibodies by *B*-cells, affect their receptors and prevent their contact with antigens); *NK*-lymphocytes (natural killers) are formed in the bone marrow from precursors of lymphoid cells; participate in non-specific cytotoxicity towards internal cellular pathogens.

The condition characterized by an increase of the lymphocyte count in blood — is absolute (over  $3000 \cdot 10^9/l$ ) or relative (over 40 %) **lymphocytosis**. Physiological lymphocytosis is observed on the 4<sup>th</sup>–5<sup>th</sup> day after birth (in their amount, lymphocytes prevail over granulocytes — «the first over cross») and lasts up to 4–5 years, when equalizing of the relative count of leukocytes and neutrophiles occurs at the level of 40–45 % («the second over cross»). Relative lymphocytosis may persist up to 8–10 years. It becomes longer in iron deficiency, rickets and hypotrophy. In preschool children the leukocyte count in norm does not exceed  $9000 \cdot 10^9/l$ , in school children —  $7200 \cdot 10^9/l$ .

Pathologic lymphocytosis is caused by immune response to an infectious or non-infectious antigen or immune stimulator. It is observed in viral infec-



tions (herpes, chicken pox, mumps, whooping cough, ring-worms, viral hepatitis, etc.) as well as in non-viral (the disease of «cat's scratches» — benign reticulosis, listeriosis, toxoplasmosis), and also in latent infections (tuberculosis, syphilis), in some endocrinopathies (thyroidism, mixedema, eunochoidism, acromegaly), in neurasthenia and other diseases of the central nervous system, in alimentary dystrophy. Medicinal lymphocytosis may occur in taking novarsenol, atophan, etc. Relative lymphocytosis is noted in enteric fever, the flue, immune agranulocytosis, mainly in carbohydrate nutrition.

The condition, when the peripheral blood contains less than  $1.5 \cdot 10^9/l$  of lymphocytes — is **lymphopenia**. If the lymphocyte count is less than  $1 \cdot 10^9/l$ , there is a marked immune deficiency. Lymphopenia develops in suppression of lymphocytopoiesis, accelerated death of lymphocytes, impairment of their migration or combination of these factors. The restriction of the lymphocyte formation processes occurs more often in protein deficiency, e. g. in fasting, in particular in kwashiorkor. A lymphocyte count decrease in the blood lower than  $1.2 \cdot 10^9/l$  is considered an absolute sign of protein deficiency in the organism, if there are no other causes of lymphopenia. Restriction of lymphopoiesis occurs in medullary insufficiency, radiation injury, taking immune depressors, hereditary mixed and *T*-cellular immune deficiency, lymphogranulomatosis, myeloid leukomoid reactions caused by the action of cytokines. Accelerated death of lymphocytes occurs in infections that affect them (lymphotropic viruses — measles, poliomyelitis, human immune deficiency virus); under the effect of cytostatic drugs, anti-lymphocytic bodies.

#### 4.5. LEUKEMIAS. GENERAL CHARACTERISTIC

Leukemia — is a systemic clonal neoplastic disease, when a mutant tumor clone originates from progenitor hemopoietic cells, it occurs primarily in the bone marrow; is manifested by unrestricted proliferation and rejuvenation of hemopoietic elements with retardation of their maturation and metaplasia of the hemopoietic tissue.

Leukemias are a variety of hemoblastoses — tumor diseases of the hemopoietic tissue. The tumor origin of leukemias is confirmed by tumor progression, fast cellular multiplication, their atypical structure, infiltrating growth, metastasizing, impairment of metabolism, cachexia and frequent death of the organism.

The etiology of leukemias is similar to the majority of malignant neoplasms. A definite role in their development is played by genetic, immunologic and environmental factors. The pathogenesis of leukemias follows the general principles characteristic of the mechanisms of tumor growth, it includes the development of anaplasia, hyperplasia, metaplasia, tumor progression, paraneoplastic syndrome.

Leukemic cells are not identical to blast cells and more mature elements, that are present in the process of normal hemopoiesis. They differ in histochemical, immunophenotypical peculiarities and in their majority in chromosomal abnormalities. The latter play the role of a central link of leukemia pathogenesis, as somatic mutations result in hyperexpression of oncogens and/or deletion of anti-oncogens.

#### 4.5.1. Classification of leukemias<sup>1</sup>

The following principles underlie the classification of leukemias:

- 1) histo- (cyto-) genesis of tumor cells (their histogenetic characteristic);
- 2) differentiation degree (maturity) of leukemic cells and the course of leukemia;

**Histologic character** of leukemic cells allows to differentiate:

- malignant immune-proliferative diseases (cellular neoplasms of a lymphoid series), they include: plasmocellular, acute lymphoblast, chronic lymphoblast, lymphocyte, prolymphocyte leukemias, etc.;
- cellular neoplasms of a myeloid series (myeloproliferative diseases-syndromes, a common feature of which is proliferation of a myeloid germ) — chronic myeloid leukemia, promyelocyte, chronic myelomonocyte and monocyte leukemias, chronic erythremia, essential thrombocytemia, etc.

By a **degree of differentiation** (of maturity) of leukemic cells, leukemias can be acute and chronic.

In acute leukemias the substrate of the tumor consists of blast cells resulting from neoplastic monoclonal proliferation of hemopoietic stem cells; over 30 % of leukemia blasts are revealed in bone marrow, in their amount they prevail in peripheral blood too, are characterized by complete retardation of maturation, maturing and differentiated forms of leukocytes are absent or considerably decreased (leukemic failure — hiatus leucemicus, particularly marked in acute myeloid leukemia). The content of *Hb* sharply falls down, irreversible anemia and hemorrhagic diathesis develop (hemopoiesis is impaired already in the beginning of the disease). Leukemia cells carry markers on their surface, which characterize definite differentiation stages of normal hemopoietic cells.

In chronic leukemias maturation of cells is partially inhibited, the tumor substrate consists of maturing and mature cells that are mainly revealed in peripheral blood, in the majority of cases anemia develops as the disease is progressing.

A slower course of leukemia is not prognostically more favorable. In many cases acute leukemias are successfully treated while chronic ones may be resistant to therapy.

Acute and chronic leukemias develop on different clonal and non-identical mutation basis. Acute leukemia does not pass into chronic one with time, as

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<sup>1</sup> Basic classifications and the most common types of leukemia are presented.

the neoplastic clone does not acquire again the lost ability for differentiation. Nevertheless chronic leukemia may transfer into an acute one.

**By the leukocyte count in peripheral blood** leukemias on this or that stage of their course are qualified as: **leukemic** (a sharp increase of the leukocyte count —  $100.0 \times 10^9/l$  and over); **sub-leukemic** (an increase of the leukocyte count up to  $100.0 \times 10^9/l$ ); **aleukemic** (the leukocyte count is not changed); **leukopenic** (the leukocyte count is decreased —  $<4 \times 10^9/l$ ).

#### **4.5.2. Acute lymphoblast leukemia (ALL)**

ALL — is a tumor developing from a precursor-cell of lymphopoiesis. It occurs rare in adults; in childhood it comprises 80 % of all forms of leukemia. Leukemic lymphoblasts squeeze out myeloid elements from the bone marrow and substitute them in peripheral blood. Blast cells with an abnormal karyotype are revealed (aneuploidy, changing of the chromosomal structure). The most important abnormality is translocation  $t(9;22)$  — Ph chromosome, that is primarily described as characteristic of chronic myeloleukemia.

The blood picture in ALL reveals anemia, thrombocytopenia, granulocytopenia, lymphoblastosis, the content of differentiated lymphocytes is considerably decreased — absolute lymphopenia. Besides, its clinical picture has no anemic, hemorrhagic, infectious-septic syndromes, paraneoplastic symptoms caused by cytokines excreted by immune cells, leukemic blasts (anorexia, emaciation, osteoporosis, pain in bones). There are also revealed lymphadenopathy, hepatosplenomegaly, meningeal phenomena, neuroleukemia.

#### **4.5.3. Chronic leukocyte leukemia (CLL)**

CLL is divided into *B*- (85 % of cases) и *T*-forms and is a genetically conditioned tumor of the immune-competent system, their peripheral organs, it is referred to lymphomas. On progressing of the disease there occurs chromosomal aberration at the level of a *B*-cell precursor, it leading to trisomy of chromosome 12 or structural impairments in chromosome 6, 11, 13 and 14. A sharp decrease or perversion of humoral immunity is common, it being revealed by a decrease of immunoglobulins in the blood, the ability to produce antibodies and interferon, as well as a decrease of resistance to bacterial and viral infections. Besides, lymphocytes are characterized by defective immune response, their ability for blast-transformation is decreased and there appear perverse immune reactions, autoimmune complications, frequent recurrent infectious complications, recurrent infections characterized by a prolonged severe course. The peripheral blood reveals an increase of the lymphocyte content (up to 80–90 %) with the presence of young forms (prolymphocytes, sometimes lymphoblasts). It is common to reveal defect lymphocytes smashed while preparing a smear (diffuse spots, remains of nuclear chromatin), defined as bodies (shadows) of Botkin-Gumprecht-Klein. They evidence increased fragility of lym-

phocytes. As the disease is developing, anemia and thrombocytopenia are progressing and ESR increases. In the development mechanism of anemia in CLL an important role is played by autoimmune hemolysis of erythrocytes and thrombocytes, shortening of their life span associated with damaging action of the enlarged spleen and absence of a compensatory reaction in response to premature death of erythrocytes revealed by increased erythropoietic activity of the bone marrow. At the stage of a blast crisis (terminal stage of CLL) the amount of blast cells in the bone marrow and in the blood sharply grows (over 30) and severe infectious complications occur.

#### **4.5.4. Acute Myeloblast Leukemia (AML)**

AML is a tumor originating from a stem cell and a precursor-cell of myelopoiesis consisting mainly of progenitor elements of a granulocyte series — myeloblasts, that considerably lost their ability to differentiate

The bone marrow reveals «blasts» with chromosomal abnormalities — deletion of a long lever of chromosome 5 or monosomia on chromosome 7.

In peripheral blood — pancytopenia, anemia with megaloblast and hyperchromous cells, resistant both to iron and trace elements and folic acid, leukemic blasts are present in the blood, in internal organs, in cerebral membranes, in gums; in the bone marrow their amount exceeds 30 %; a leukemic failure, normally maturing clones are squeezed out by leukemic cells from the bone marrow and peripheral blood. In the cytoplasm of blasts and more mature cells, Auer's bodies are revealed — abnormal rod-like forms of azurophilous granules.

Clinical manifestations: fatigue, pallor, loss of weight, breathlessness; vulnerability to severe bacterial and fungal infections, pains in bones and joints, organomegaly (due to infiltration of leukemic cells into organs), fever, purpura, softening of thoracic and other bones (hyperplasia of leukemic cells inside the bone marrow), hemorrhages, manifestations of DBC-syndrome; meningeal syndrome, CNS impairments, intracranial hemorrhages, leukostases (vascular occlusions in various organs associated with aggregation of leukocytes in leukemic forms) and other hemorrhagic, infectious-septic and hypoxic manifestations.

#### **4.5.5. Chronic myeloid leukemia (CML)**

CML — is a tumor clonic disease of the hemopoietic tissue developing in transformation of a stem cell or a precursor-cell of myelopoiesis. On the level of the precursor-cell occurs reciprocal translocation between chromosomes 9 and 22 —  $t(9-22)$ , shortening of a long lever of the 22<sup>nd</sup> chromosomal pair that is denoted as  $Ph^1$ -chromosome (Philadelphia). In 5–8 % of patients variation translocations are revealed, when an exchange between chromosome 22 and any other (4, 12, 19, 21) takes place.

The expanded stage of the process is characterized by overgrowing of the white germ of the myeloid tissue not only in the bone marrow, but also in

the adipose, connective tissues, uterus, etc., as well as substitution of fat of tubular bones for the myeloid tissue.

Hematological pattern: leukocytosis, a shift of the leukocyte formula to the left till blasts (10 %) with the presence of all forms of granulocytes, basophilia, eosinophilia (basophile-eosinophile association), thrombocytosis or thrombopenia, increasing of ESR; normochromous anemia with a great number of normoblasts. In the basis of its pathogenesis lies squeezing out, suppression of an erythroblast germ of the hemopoietic tissue with a leukoblast one (metaplastic anemia). The state of the red blood («leukemia barometer») serves an indicator of the process severity, the range of leukemic infiltration.

Clinical manifestations of CML are general weakness, the feeling of heaviness and pain in the left hypochondrium, subfebrile temperature, intoxication symptoms and hemorrhagic syndrome. There may be impairments of the cardio-vascular, respiratory system, liver, spleen, gastro-intestinal tract, pancreas, urinary system with corresponding symptoms. In high basophilia itching, periodic feeling of fever, diarrhea may occur; it is associated with an increased content of histamine in the blood that is produced by basophiles. Infiltrates of blast cells may localize in bones causing pathologic fractures. The immunologic examination of patients reveals a decrease of *T*-helpers, regulatory lymphocytes, depression of phagocyte activity of neutrophils.

The thermal stage is characterized by: high temperature, progressing emaciation, fast enlargement of the spleen. In essential splenomegaly an infarction of the spleen develops that is accompanied by sharp pains irradiating to the back, as well as nausea and vomiting. The central nervous system impairment is manifested by neuroleukemia, agonizing pains and pareses, a blast crisis develops. According to morphological, cytochemical and immunophenotypical characteristics of blast cells, blast crises are of different varieties, more often it is myeloblast or lymphoblast crisis, less frequently — promyelocyte, monoblast, myelomonoblast, erythroblast and megakaryoblast one.

#### **4.5.6. Chronic myelomonocyte leukemia (CMML)**

The blood picture of CMML is characterized by: absolute monocytosis (over  $1 \cdot 10^9/l$ ), absolute or relative neutropenia; pathologic shapes of neutrophils (hypo- or hypersegmentation of nuclei, dark granules in the cytoplasm — Chediak–Higashi cells); hypogranulated basophiles and eosinophiles; pathologic shapes of monocytes («jagged» outlines of cells); до 5 % of blast cells, thrombocytopenia, gradually progressing anemia. In the bone marrow — up to 20 % of monoblasts, the count of monocytes, promyelocytes and myelocytes is increased. The cytochemical examination of leukemic blasts reveals a positive reaction to peroxidase and high activity of non-specific esterase. According to cytogenetic examinations the cells of a neoplastic clone are characterized by the following features: deletion of a short lever of chromosome 12 (12p), dele-

tion of a long lever of chromosome 7 (7q), trisomy of chromosome 8. The basic clinical manifestations of the disease are: recurrent infectious complications, autoimmune syndrome, splenomegaly, hepatomegaly, hemorrhages, weakness and breathlessness.

#### **4.5.7. Vaquez' disease (erythremia)**

Erythremia (true polycythemia) — is a chronic leukemia with impairment at the level of a stem cell or a precursor-cell of myelopoiesis. It is characterized by total hyperplasia of medullary cells with abnormal tumor proliferation of erythroid, myeloid and megakaryocyte germs (panmyelosis), mainly of an erythroid series (primary absolute erythrocytosis) — the erythrocyte count irreversibly increases, while the level of erythropoietine in the blood and urine is low. The disease is considered to have two clones of erythroid precursors: that is dependent on erythropoietine and an independent one, erythroid colonies of which grow without erythropoietine. There are revealed somatic mutations of a long lever of chromosome 22, aberrations, aneuploidies, etc. However the characters of differentiation in erythroid cells are preserved.

The onset of the disease is gradual, the skin and mucous membranes get a cherry-red color with a cyanotic shade, it is marked by dizziness, headaches, noise in the ears, injection of vessels of the retina and conjunctiva («rabbits' eyes»), BP sharply elevates. The symptoms are explained by increasing of the circulating blood mass, increased number of blood cells (polycytemic hypervolemia), that causes blood viscosity, slowing down of the blood flow, impairment of hemodynamics. There develops hypoxia, hepatomegaly and splenomegaly.

The hemogram reveals erythrocytosis (up to  $6.0-12.0 \cdot 10^{12}/l$ ), hypochromia (high velocity of iron consumption, decreasing of its reserves), reticulocytosis, the hematocrit index is 60–80 %; neutrophile leukocytosis with a shift of the leukocyte formula to the left, thrombocytosis with giant shapes. The content of hemoglobin increases up to 180–200 g/l; there is noted polychromasia, anisocytosis, basophile granularity of erythrocytes, normoblastosis, toxic granularity of neutrophiles. ESR is reduced. There occur transformation of the yellow bone marrow into the red one; the myelogram shows increasing of the elements number of an erythrocyte, granulocyte and megakaryocyte series.

Basic complications: a thrombohemorrhagic syndrome, coronary and cerebral ischemia, strokes, central paralyzes, blindness (thrombosis of retinal veins). Thromboses of abdominal veins give symptoms of «acute stomach». In the terminal stage erythremia usually transforms into myelofibrosis and pancytopenia develops.

#### **4.5.8. Essential thrombocytemia**

Essencial thrombocytemia (synonyms: primary thrombocytemia, idiopathic thrombocytemia, hemorrhagic thrombocytemia, chronic megakaryocyte

leukemia) — is a chronic myeloproliferative disease of a clonal neoplastic origin with impairment at the level of a stem cell. Its course is accompanied by predominant proliferation of megakaryocytes and intense formation of thrombi. It is manifested by an increase of the thrombocyte count (up to  $800.0 \cdot 10^9/l$  and over), leukocytosis, anemia, microcirculatory disturbances, thrombosis of arteries and veins, thromboembolic complications, hemorrhages, as well as ischemia of the brain, angina pectoris, attacks of sharp pains in toes and fingers, their edema, feeling of fever, reddening of the skin due to increasing of thrombocytes aggregation in arterioles that may be complicated by gangrene of fingers.

#### **4.5.9. Chronic erythromyelosis**

The disease is characterized by hyperplasia of medullary red germ cells. The impairment occurs at the level of a precursor-cell of myelopoiesis. There develops progressing normo- or hyperchromous anemia with megaloblastic features, appear myelocytes, promyelocytes and myeloblasts. The number of erythroblasts, myeloblasts and undifferentiated medullary blast cells increases. Erythromyelosis is terminated by a blast crisis.

#### **4.5.10. General impairments in the organism in leukemia.**

The following syndromes develop in leukemia, often with a fatal outcome: anemic (depression of the medullary erythroid germ); hemorrhagic (bleeding from the nose, gums, intestines; hemorrhages into vital organs) — are caused by an intensity decrease of thrombocyte production; infectious (functional abnormality of leukemic leukocytes — reducing of phagocytosis ability, depression of antibodies synthesis); metastatic (functional impairment of organs and systems due to the appearance of leukemic neutrophils there); intoxication (overflowing of the organism with toxic products formed in breakdown of leukemic cells); osteoarthropathic (tenderness of bones, joints caused by tumor hyperplasia of the medullary hemopoietic tissue).

### **4.6. LEUKEMOID REACTIONS**

Leukemoid reactions are reactive, to some degree known functional conditions of the hemopoietic apparatus, lymphatic and immune systems of the organism, occurring on the background of various diseases. LR — is not an independent disease, but it presents changes of the peripheral blood (leukocytosis and changing of the leukocyte formula) and organs of hemopoiesis, they are similar to leukemia and other tumors but don't transform into them.

The basic groups of leukemoid reactions are leukemoid reactions of a myeloid and lymphoid type.

Reactions of a myeloid type may be of two kinds:

1) with the blood picture corresponding to that one in chronic myelosis; they develop in: infections — sepsis, scarlet fever, erysipelas, purulent process-

es, diphtheria, croupous (membranous) pneumonia, tuberculosis, dysentery, etc.; exposure of ionizing radiation; cranial injuries; intoxications (uremia, CO poisoning); metastases of malignant tumors into the bone marrow; lymphogranulomatosis;

2) eosinophilous type («essential eosinophilias»), develop in allergic processes either in diseases with an allergic component or in helminthes and parasitic diseases characterized by the appearance of a great number of eosinophiles (up to 90 %, in leukocytosis up to  $100 \cdot 10^9$ ), hypersegmentation of eosinophile nuclei may be observed.

Reactions of the lymphoid type are divided into the following kinds:

1. With predominance of lymphocytes with atypical morphology, develops in infectious mononucleosis (Filatov-Pfeifer's disease). It is a disease of a viral etiology. It starts sharply with sharp elevation of temperature that persists at 39–39.5 °C during the day. Sometimes the fever is preceded by prodromal phenomena: malaise, muscular pains, dizziness, systemic enlargement of lymphatic nodes, that reach their maximum sizes by the 4<sup>th</sup>–6<sup>th</sup> day of the disease; in 10–15 days they become smaller, but their slight enlargement and tenderness may persist for some weeks, sometimes months; the spleen is enlarged, quinsy with necrotic changes develops. At the peak of the disease leukocytosis develops ( $10.0$ – $25.0 \cdot 10^9/l$  of leukocytes). The leukogram reveals up to 50–70 % of lymphocytes and a high percentage of monocytes (from 12 to 40–50 %). Atypical lymphocytes usually appear — «lymphomonocytes» (the cells greater than lymphocytes but smaller than monocytes, they have a monocyte shape of the nucleus and intensively basophilic cytoplasm). «Lymphomonocytes» are modulated *T*- and *NK*-lymphocytes, that enter the blood flow, when *B*-lymphocytes get infected. There may be observed moderate anemia, sometimes slight thrombocytopenia and neutropenia. The prognosis is favorable; fatal outcomes occur seldom.

2. With predominance of typical lymphocytes (infectious lymphocytosis). It develops in acute viral and bacterial infections; is characterized by leukocytosis with absolute lymphocytosis, an increase of lymphoblasts and prolymphocytes in bone marrow (they are absent in peripheral blood).



## Chapter 5

### THE THROMBOCYTE SYSTEM AND ITS IMPAIRMENTS

In norm the human blood contains  $150.0\text{--}450.0 \cdot 10^9/l$  of thrombocytes. Their life span is 8–11 days.

#### 5.1. THROMBOCYTOPOIESIS, FUNCTIONS OF THROMBOCYTES

Thrombocyte formation is accomplished in bone marrow on a megakaryoblast type of hemopoiesis. The first morphologically identified cell of this series is a *megakaryoblast* — 20  $\mu\text{m}$  in size, the cytoplasm is basophilic, the nucleus occupies most of the cell, is stained in a red-violet color, has a rough structure, contains one-two nuclei.

A *promegakaryocyte* — occurs in the result of some endomitoses. The cytoplasm is basophilic without granules. The nucleus is polymorphous with a rough structure.

Then a *megakaryocyte* is formed (60–120  $\mu\text{m}$ ). The cytoplasm of the cell is stained in a reddish-lilac color, has plentiful azurophilic granularity. The nucleus is polymorphous.

*Thrombocytes* are formed in the cytoplasm of megakaryocytes and are separated from them. As a result of separation of thrombocytes the nucleus of megakaryocytes, deprived of the cytoplasm, breaks into separate fragments and is removed by phagocytosis. A thrombocyte or a platelet is a polymorphous cytoplasmatic formation without the nucleus 3–4  $\mu\text{m}$  in size, surrounded by a membrane, has a central zone — a granulomer, consisting of 5–20 azurophilic granules, and a peripheral zone — a hyalomer.

Thrombocytes circulating in blood have an oval or rounded shape, smooth surface, while activated ones have a stellate shape and filament processes — pseudopodias.

The basic physiologic role of thrombocytes — participation in hemostasis — is accomplished due to their following functions: angiotrophic — provision of vital activity and repair of endothelial cells («feeders of endothelium») and sustaining the normal structure and functions of vascular cells of the microcirculatory channel; adhesion-aggregation — participation in primary hemostasis by forming a thrombocyte cork or a white embolus; angiospastic — spasm sustenance of injured vessels due to the formation of serotonin, catecholamines, *B*-thrombomoduline; coagulatory-platelet — participation in blood coagulation and in regulation of fibrinolysis — excretion of thrombocyte factors (TF); repair — growth factors of thrombocytes stimulate multiplication and migration of smooth muscular cells and endotheliocytes, and thus participate in pathogenesis of atherosclerosis, ischemic heart disease, in transplant rejection, development of tumor metastases.

## 5.2. TYPICAL KINDS OF IMPAIRMENTS AND REACTIVE CHANGES IN THE THROMBOCYTE SYSTEM

There are the following basic groups of typical impairments and reactive changes in the thrombocyte system: an increase of the thrombocyte count in a blood volume unit over the norm — thrombocytosis; a decrease of the thrombocyte count in a blood volume below the normal level — thrombocytopenia; changing of functional properties of thrombocytes (adhesion, aggregation, releasing) — thrombocytopathy; a combination of the mentioned deviations.

### 5.2.1. Thrombocytoses

**Thrombocytoses** are divided into primary (tumor, essential) and secondary (reactive and developing after splenectomy).

*Primary thrombocytoses* occur as a result of a clonal defect of hemopoietic stem cells. They may be either a symptom of myeloproliferative diseases (chronic myeloleukosis, erythremia, myelofibrosis, acute megakaryoblastic leucosis, etc.), or an independent nosologic form (essential thrombocytemia). (see part 4.5.8.).

*Secondary — reactive thrombocytoses* — are qualitative benign changes without any impairments of morphology and functions. Their genesis is not associated with the impairment of hemopoietic cells. They occur due to the effect of medicinal preparations (adrenalin, nor-adrenalin, vincristine, vinblastin), in massive hemorrhages, traumas, surgical interventions, acute and chronic infections, diseases of the connective tissue, hemolysis, chronic deficiency of iron in the organism, physical exertion, stress, or after splenectomy.

There are also *absolute* (true, proliferative) thrombocytoses characterized by an increase of the thrombocyte count in blood due to their increased formation, and *relative* (false, non-proliferative). The latter are not accompanied by an increase of the total thrombocyte count in blood (redistribution and hemococentration thrombocytoses).

Thrombocytoses may have both compensatory-adaptive and pathogenic **significance**.

*The compensatory-adaptive significance of reactive thrombocytoses* is in the formation of a thrombocyte clot and later of a thrombus, e.g. in breaking the vessels integrity (after trauma, operative interventions or blood loss).

*Pathogenic significance of thrombocytoses* is caused by coagulation activation of blood proteins and tromboformation that is accompanied by the impairment of microcirculation in tissues, for example, in megakaryoblast leucosis.

### 5.2.2. Thrombocytopenias (TP)

Thrombocytopenias may be independent diseases or symptoms of various diseases, hereditarily conditioned or acquired. They develop due to: suppres-

sion of the thrombocyte germ; activation of the thrombocytes destruction process; involvement of thrombocytes into the process of generalized thrombus formation; elevated deponing of thrombocytes in the spleen; massive blood loss.

*Hereditary TP* are divided into: those that are caused by insufficient number of megakaryocytes in bone marrow (Fankoni's syndrome, cyclic amegakaryocyte TP, etc.); caused by ineffective thrombocytopoiesis due to a defect of thrombopoietine synthesis or dystrophy of megakaryocytes (syndrome of «grey» thrombocytes, abnormality of May-Heggline, etc.).

*Acquired TP* are: medullary TP caused by an intensity decrease of the process of thrombocyte production in bone marrow: hypo- and aplasia of hemopoiesis, effect of ionizing radiation, chemical substances — benzole, uretan, antibiotics, cytostatic substances, alcohol; substitution of bone marrow for tumor tissue (leukemias, lymphomas, metastases of solid tumors); inefficient thrombopoiesis (B<sub>12</sub>-folic-deficient anemias); caused by increased extramedullary destruction of thrombocytes: immune TP, (haptenuous, hetero-immune, iso-immune), autoimmune (idiopathic, in lymphoproliferative diseases) and non-immune TP (in leukemias, tuberculosis, sarcoidosis, malaria, enteric fever, etc.); the result of mechanic lesion of thrombocytes (catheters, prostheses of heart valves, extracorporal circulation); caused by increased consumption of thrombocytes: coagulopathies of consumption (Hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura — Moschcowitz's disease, hemorrhagic vasculitis, etc.), thrombophilias; peripheral ones occurring due to dilution and redistribution.

Thrombocytopenias are manifested in peripheral blood by a decrease of the thrombocyte count and enlargement of their sizes in the normal erythrocyte count, *Hb* and leukocytes; by prolongation of bleeding; decrease of the retraction degree of a blood clot; by the development of a hemorrhagic syndrome.

### **5.2.3. Thrombocytopathies**

Thrombocytopathies may be hereditary (primary) that develop in gene defects: (thrombasthenia of Glanzmann, Willebrand disease, Bernard-Selye disease).

### **5.2.4. Glanzmann's thrombasthenia**

The disease is inherited autosomally-recessively, is revealed already in early childhood and is characterized by a petechial-ecchymous type of bleeding, disposition to hemorrhages from mucous membranes (nasal, uterine bleedings, hemorrhages into the sclera and retina of the eye), prolonged bleedings after tooth removal of laryngological operations.

The development of the thrombocyte dysfunction is caused by the absence or membrane receptor defect to fibrinogen and glycoproteins IIb–IIIa. It results

in a sharp intensity decrease of the binding process of fibrinogen with the thrombocyte membrane causing the impairment of thrombocyte aggregation.

### **5.2.5. Willebrand disease**

The deficiency or functional abnormality of Willebrand factor (WF) underlies the development of the disease. It is inherited autosomally-dominantly with incomplete penetrance or (less frequently) — autosomally-recessively.

The deficiency and/or defect of the WF structure causes the impairment of thrombocyte adhesion to collagen of the vascular wall and intensity decrease of the FV–FVIII complex formation, the thrombocyte count being normal.

The clinical picture of the disease is diverse, it depends both on phenotypical manifestation of a pathologic gene and on the physical status of the organism. The deficiency and/or defect of the WF structure causes the impairment of both vascular-thrombocyte and coagulatory hemostasis. It is manifested by ecchymous, less frequent — hematous hemorrhages, menorrhagias, bleeding mucous membranes. Surgical interventions are at risk of profuse bleedings.

### **5.2.6. The syndrome (disease) of Bernar-Sulje, the syndrome of giant thrombocytes**

In this disease the thrombocyte membrane has no specific glycoprotein interacting with FW-VIII, FV, FIX and ristocetine; the content of sialic acids being increased and the electric charge being decreased. It results in the impairment of adhesive properties of thrombocytes. The disease is inherited autosomally-recessively, is characterized by shortening of thrombocytes life span in their normal process of reproduction in bone marrow followed by the development of moderate thrombocytopenia.

The basic morphological criteria of the disease are the presence of giant thrombocytes in the blood; they reach 6–8  $\mu\text{m}$  (in norm 2–4  $\mu\text{m}$ ).

The clinical picture is characterized by petechial bleeding, the severity of which varies greatly — from relatively light and latent forms to severe and even fatal cases depending on the content of abnormal thrombocytes.

Acquired (symptomatic) secondary thrombocytopathies occur in: diseases and syndromes (tumors, DIC-syndrome, heart defects, uremia, immune thrombocytopenias, diffuse diseases of the connective tissue, hepatic and renal diseases, megaloblast anemias, acute leukemias, myeloproliferative diseases) and under the effect of medicinal preparations (aspirin, curantil, non-steroid anti-inflammatory drugs, etc.).

The pathogenesis of thrombocytopathies may have the following forms: the impairment of synthesis and accumulation of biologically active substances in granules of thrombocytes; the impairment of degranulation and releasing of thrombocyte factors into the blood plasma; the impairment of the structure and properties of thrombocyte membranes.

Thrombocytopathies are revealed by a hemorrhagic syndrome, disturbances of microcirculation (capillary-trophic insufficiency), dystrophies, erosions, ulcerations, changes of thrombocyte properties, defects of thrombocyte granules — absence or decrease of their count (for example, in the syndrome of «grey» thrombocytes), anemic syndrome — general weakness, pallor, dizziness, etc.

## Chapter 6

### THE SYSTEM OF HEMOSTASIS AND ITS IMPAIRMENTS (HEMOSTASIOPATHIES)

**Hemostasis** — is the biological system providing prevention and arrest of bleeding, restoration of the vascular wall as well as preservation of a liquid blood content, the local character and reversibility of thrombosis, sufficient blood supply of the organs. There are **three basic components of hemostasis**: vascular — hemostatic mechanisms of the vascular wall providing the spasm of an injured vessel, triggering the processes of coagulation and thrombus formation; cellular (thrombocyte-leukocyte) — the formation of a white thrombus; plasmatic: a) the coagulation system producing fibrin necessary for the formation of red and mixed thrombi; б) the anticoagulation system consisting of the anticoagulation and fibrinolytic system.

All three components of hemostasis are triggered simultaneously at the moment of the vessel injury. The main triggering mechanism for the cellular and plasmatic components is a contact of blood with an injured vascular wall or any other negatively charged polymer molecules and surfaces.

There is: primary hemostasis — vascular-thrombocyte, where the leading role is played by thrombocytes and microvessels; it means the arrest of bleeding from fine vessels, the diameter of which doesn't exceed 100  $\mu\text{m}$ , and secondary — coagulatory, where plasmatic factors are of primary significance, fibrin clots are formed and the thrombus is fixed in vessels; there occurs «struggle» with blood loss, when greater vessels are injured, their diameter exceeds 100  $\mu\text{m}$ . In the accomplishment of the secondary hemostasis all components of the chain take part, mainly a fibrinous one.

The imbalance in the system of hemostasis may be caused by pathology: of thrombocytes, (thrombocytoses, thrombocytopenias and thrombocytopathies); of plasmatic systems: coagulation system, anti-coagulation system, the system of fibrinolysis; vascular wall (vasopathies).

The basic manifestations of this pathology are: a hemorrhagic syndrome (hemorrhagic hemostasiopathy), manifested by bleeding — hemorrhagic diathesis and thrombotic syndrome (thrombotic hemostasiopathy), manifested by thrombus-formation of various localization, as well as their combination — thrombohemorrhagic hemostasiopathy (DBC-syndrome).

#### 6.1. VASCULAR-THROMBOCYTE HEMOSTASIS, ITS IMPAIRMENTS

In case the wall of a microvessel is damaged, thrombocytes are stuck to damaged parts of the vascular wall (adhesion), undergo structural-functional changes excreting the content of their granules (releasing reaction), get stuck to each other (aggregation) leading to fast formation of a thrombocyte cork and

arrest of bleeding in microvessels. Simultaneously the spasm of the damaged vessel occurs.

### **6.1.1. Immune thrombocytopenia of consumption — immune thrombocytopenic purpura (ITP)**

ITP — is a group of diseases, when the thrombocytes life span decreases due to the action of antibodies.

There is an autoimmune and haptenuous **form of ITP**.

*Autoimmune forms* may be: symptomatic (in systemic diseases of the connective tissue, chronic lympholeukemia, lymphogranulomatosis, lymphomas, etc.) and idiopathic (without any association with any previous diseases), for example, Werlhof's disease characterized by chronic course, obligatory presence of megakaryocytosis in bone marrow, giant thrombocytes and antithrombocyte bodies.

*Haptenuous forms* are related to the action of some medicinal preparations (quinine, quinidine, preparations of gold, etc.).

**The clinical picture is characterized** by multiple petechial eruptions, bruises of various shapes on the skin, cerebral hemorrhages are possible

## **6.2. COAGULATORY HEMOSTASIS, ITS IMPAIRMENTS**

The coagulatory hemostasis (secondary) is realized with involvement of the coagulating, anti-coagulating and fibrinolytic blood systems.

The coagulating blood system is a number of interconnected reactions involving proteolytical enzymes, plasmatic proteins (coagulation factors) and providing the formation of a permanent thrombus.

### **6.2.1. The pathology of the coagulation system (coagulopathies)**

Coagulopathies may be hereditary and acquired.

**Hereditary coagulopathies** — are diseases caused by deficiency of factors VIII and IX, and are the most common hereditary coagulopathies (over 95 % of cases). The deficiency of factors VII, X, V, XI comprises up to 1,5 %; the deficiency of other factors (XII, II, I, XIII) occurs very rare (single cases).

*Hemophilia A (deficiency of factor VIII)*. The disease is inherited recessively, linked with X-chromosome. It is a disease of male sex (10 cases per 100 thousand of men).

The deficiency of factor FVIII causes a sharp increase of the time for the formation of a prothrombin complex that is accompanied by prolonged, practically continuous bleeding in slight lesion of vessels (biting of the tongue, contusions, etc.). Hemophilia A is characterized by hematomous bleeding.

In a slight form of the disease bleedings are possible only in severe injuries or operative interventions. The course of the disease is subclinical and it is often not diagnosed. In severe or very severe forms (2 % and less, 1 %, accord-

ingly) develop recurrent hemorrhages into large joints (hemarthroses), causing ankilization; large inter- and intramuscular, subperitoneal hematomas with subsequent destruction of soft tissues, severe and frequent spontaneous bleedings, persistent recurrent gastro-intestinal and renal bleedings.

*Hemophilia B (Christmas disease, FIX deficiency)*. The disease is inherited recessively, linked with *X*-chromosome. This defect causes considerable inhibition of a prothrombinase complex formation resulting in the development of hematomous bleeding.

The clinical picture of hemophilia *B* is identical to that of hemophilia.

*Hemophilia C (deficiency of XI factor)* is inherited autosomally-recessively; heterozygotes have slight bleedings, while in homozygotes with FXI deficiency the complications associated with bleedings are not many. But in lesions and surgical interventions the occurrence of severe bleedings with formation of hemarthrosis and hematomas is not excluded.

*Parahemophilia (FV deficiency)* is inherited autosomally-dominantly. The disease is characterized by a hemorrhagic syndrome, the expressivity of which depends on the deficiency degree of FV in plasma. The most severe bleeding is observed in patients, the FV level of whom is less than 2 %. This disease is marked by petechiae, ecchymoses, bruises, bleedings from the nose, gums, gastro-intestinal tract, menorrhagias. The patients with expressed forms of the disease often have prolonged bleedings after removal of teeth, traumas and lacerations.

### **6.2.2. Acquired coagulopathy (disseminated intravascular coagulation, DIC-syndrome)**

DIC-syndrome — is a non-specific general pathologic process characterized by generalized activation of the hemostasis system, when disagreement of regulatory systems of the blood aggregate condition occurs.

The etiological factors of the disease are: generalized infections, septic conditions; shock of any origin; extensive surgical interventions; malignant tumors; extensive tissue lesions, tissue embolism, burns; immune, allergic and immune-complex diseases; massive blood losses, transfusions; poisonings with hemocoagulating venoms, chemical and vegetative substances, intravascular hemolysis of any origin; acute hypoxias, hypothermia, hyperthermia with dehydration.

DIC-syndrome is accompanied by the impairment of both vascular-thrombocyte and coagulation types of hemostasis.

The following mechanisms underlie its pathogenesis: systemic damage and abnormality of vascular endothelium; activation of the coagulation system and thrombocytes; primary or secondary depression of the anti-coagulatory system.

The clinical picture of DIC-syndrome is marked by:



– in the 1<sup>st</sup> stage — the symptoms of the basic disease and signs of a thrombohemorrhagic syndrome (signs of generalized thrombosis prevail); hypovolemia, impairment of microcirculation, dysfunction and dystrophic changes in organs;

– in the 2<sup>nd</sup> stage appear the symptoms of polyorgan damage and blockade of the microcirculatory system of parenchymatous organs, hemorrhagic syndrome;

– in the 3<sup>rd</sup> stage the above impairments are supplemented by the signs of polyorgan insufficiency (acute respiratory, cardio-vascular, hepatic, renal, intestinal paresis) and metabolic impairments (hypokalemia, hypoproteinemia, metabolic acidosis, alkalosis), as well as anemic syndrome, hemorrhagic syndrome on a mixed type (petechiae, hematomas, bleeding, hemorrhages into vital organs);

– in the 4<sup>th</sup> stage (in favorable outcome) the basic vital functions and hemostasis factors gradually come into norm.

DIC-syndrome may be: swift (from some minutes to some hours or 1 day); acute (1–10 days); subacute (up to 1 month); chronic (over 1 month); recurrent (wave-like).

### 6.3. PATHOLOGY OF THE ANTI-COAGULATORY SYSTEM

The factors of the anti-coagulatory system include inhibitors of proteinases. The main of them are: antithrombin III (heparin factor I), heparin co-factor II, etc.

The deficiency of these or those mentioned factors cause functional impairments of the anti-coagulatory system. This pathology may be hereditary or acquired.

Hereditary factor deficiency of the anti-coagulatory system is clinically revealed by the development of venous thrombosis, resistant to anti-coagulation therapy. Venous thrombosis of lower extremities in the majority of patients with hereditary factor deficiency of the anti-coagulatory system results in thromboembolism of the pulmonary artery.

The acquired impairments include **the antiphospholipid syndrome (APS)**, when antiphospholipid antibodies (anti-PL) appear in the blood. There is a primary and secondary APS.

The primary APS takes place in the absence of any diseases. It occurs seldom. More often the secondary APS develops, it occurs in autoimmune systemic diseases of the connective tissue (systemic erythema centrifugum, rheumatoid arthritis), malignant growths, AIDS and other viral and bacterial infections.

The clinical picture is manifested by venous thrombosis (thrombosis of deep veins of lower extremities, pulmonary embolism and thrombosis of renal or hepatic veins). Arterial thrombosis is revealed by the damage of arteries, particularly of coronary, cerebral, rarely — peripheral. There develop transient is-

chemic attacks, single or recurrent cerebral infarctions, the vision worsens, temporary blindness occurs; there is noted vasculitis, rash, arthralgias, migraine, retina exfoliation, endocarditis with affection of the mitral valve. In pregnancy a risk of miscarriage is high.

#### **6.4. HEMOSTASIS IMPAIRMENTS OF THE VASCULAR (VASOPATHIES) AND MIXED GENESIS**

There are hereditary and acquired vasopathies.

##### **6.4.1. Osler's disease (hereditary hemorrhagic teleangiectasia)**

The most common hereditary vasopathy is inherited on the autosomal-dominant type of various penetrance. The decreased content of collagen in the subendothelial layer of the vascular wall causes focal thinning and dilation of the microvessels lumen and abnormal vascular thrombocyte hemostasis. In such cases the causes of bleeding are low resistance, vulnerability of the vascular wall, dysfunction of the endothelium at the sites of angioectasia as well as the impairment of the thrombocyte aggregation function.

Characteristic skin manifestations: teleangiectasias as spots of irregular shape, vascular bundles, bright-red round or oval nodes. They begin forming by the 6<sup>th</sup>–10<sup>th</sup> year of life at wings of the nose, mucous membranes of the nose, lips, tongue and the skin of the hairy part of the head. With age their number and degree of their prevalence increases, bleeding occurs more often and in a more severe form. On mucous membranes of internal organs appear recurrent nasal, pulmonary-bronchial, gastro-intestinal bleedings from teleangiectasias, arteriovenous aneurisms are formed in the vessels of the lungs, liver, kidneys and spleen. The following is also characteristic: posthemorrhagic anemia developing as a result of persistent bleedings from teleangiectasias of the mucous membranes and hemorrhages into internal organs. There are marked: abnormality of the mesenchymal tissues, that is revealed by increased elasticity of the skin («rubber skin»), weakness of the ligament apparatus (habitual dislocations, prolapsing of heart valves). On the part of the blood, there is revealed the picture of posthemorrhagic anemia, moderate hypercoagulation, thrombocytosis; in multiple teleangiectasias — thrombocytopenia.

##### **6.4.2. Schönlein's disease (acquired hemorrhagic immune microthrombovasculitis)**

It is one of the most common hemorrhagic diseases. Multiple microthrombovasculitis affecting vessels of the skin and internal organs underlies its development. The disease more often occurs in childhood. It may be stimulated by infectious (viral, bacterial, more often streptococcal infections) and non-infectious factors (vaccination, medicinal drugs, food allergens, parasitic inva-

sions, cold). An immune-complex inflammation of the vascular wall lies in the basis of pathogenesis. Immune complexes are fixed in the walls of vessels and activate the complement system, destruction processes of the vascular wall revealed as hemorrhagic rash (hemorrhagic syndrome) and intravascular coagulation of blood (thrombotic syndrome). Simultaneously the process of fibrinolysis is inhibited.

The clinical picture of the disease is characterized by: *skin manifestations*: symmetric affection of extremities, superficial extensors of the arms, buttocks with papular-hemorrhagic rash, rising over the skin surface (palpated purpura), that may be complicated by central necroses and crusting; *arthral syndrome*: pains of various intensity in large joints (knee, ankle); *abdominal syndrome*: severe, permanent or colicky pains in the abdomen due to hemorrhages into the intestinal wall, hemorrhages into the subserous layer and mesentery, that may be accompanied by vomiting with blood, melena, appearance of fresh blood in feces, such complications as invagination, perforation of the intestines, peritonitis; *renal syndrome*: develops like acute or chronic glomerulonephritis. The development of nephritic syndrome is possible; *neurotic manifestations* (headaches, meningeal symptoms, epileptiform seizures); *pulmonary manifestations* — affection of pulmonary vessels causing a severe bleeding (sometimes fatal).

The blood reveals moderate neutrophile leukocytosis with a shift of the leukocyte formula to the left, in profuse bleedings — the picture of acute posthemorrhagic anemia, the thrombocyte count is increased or in norm, ESR is accelerated, a shift to hypercoagulation. The immunologic examination reveals elevation of the level of circulating immune complexes and *IgA*.

The coagulogram parameters: a shift to hypercoagulation: the content of fibrinogen and Willebrand factor are increased, the immunologic examination revealed elevation of the level of circulating immune complexes and *IgA*; in children an increase of antistreptolysine O titer is often revealed.

Table 3

**Blood parameters in norm**

Parameters	System SI	Extra systemic units
Erythrocytes: – female – male	$(3.7-4.7) \times 10^{12}/l$ $(3.9-5.1) \times 10^{12}/l$	3.7–4.7 million per 1 $\mu l$ 3.9–5.1 million в 1 $\mu l$
Hemoglobin ( <i>HGB</i> ): – female – male	120.0–40.0 g/l 130.0–160.0 g/l	12.0–14.0 g% 13.0–16.0 g %
Hematocrit ( <i>HCT</i> ): –female – maley мужчин	0.36–0.42 0.40–0.48	36–42 % 40–48 %
Mean erythrocyte volume (mean corpuscular volume — <i>MCV</i> ) <i>MCV = HCT: RBC</i>	80–100 phl ( $10^{-15} l$ )	80–100 $\mu m^3$

Parameters	System SI	Extra systemic units
Mean hemoglobin content per erythrocyte (mean corpuscular hemoglobin — <i>MCH</i> ) $MCH = HGB : RBC$	$25.4-34.6 \times 10^{-15}$ kg/cell	25,4–34,6 pg/cell*
Mean hemoglobin concentration per erythrocyte (mean corpuscular hemoglobin concentration — <i>MCHC</i> ) $MCHC = HGB : HCT$	0.3–0.38 kg/l	30–38 /dl* 30–38 %
Erythrocytes distribution width over the volume (red cell distribution width — <i>RDW</i> ) — anisocytosis factor	11.5–14.5 %	1.5–14.5 %
Color factor	0.8–1.0	0.8–1.0
Reticulocytes	0.2–1.0 %	20–10.0 ppm
ESR: – female – male	1–15 mm/h 1–10 mm/h	1–15 mm/h 1–10 mm/h
Leukocytes	$4.0-9.0 \times 10^9/l$	4.0–90 000 per 1 $\mu$ l
Neutrophiles: – young – rod nuclear  – segmentated	0–1 % 1–6 % $0.040-0.300 \times 10^9/l$ 47–72 % $2.000-5.500 \times 10^9/l$	0–1 % 1–6 % 40–300 per 1 $\mu$ l 47–72 % 2000–5500 per 1 $\mu$ l
Eosinophiles	1.0–5 % $0.040-0.300 \times 10^9/l$	1.0–5 % 40–300 per 1 $\mu$ l
Basophiles	0–1 % $0-0.0065 \times 10^9/l$	0–1 % 0–65 per 1 $\mu$ l
Lymphocytes	19–37 % $1.200-3.000 \times 10^9/l$	19–37 % 1200–3000 per 1 $\mu$ l
Monocytes	3–11 % $0.09-0.6 \times 10^9/l$	3–11 % 90–600 per 1 $\mu$ l
Thrombocytes	$150.0-450.0 \times 10^9/l$	150–450 000 per 1 $\mu$ l

\* — the most common used dimension of the parameter.

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Учебное издание

**Ленова** Елена Васильевна  
**Чантурия** Андрей Владимирович  
**Висмонт** Франтишек Иванович  
**Жадан** Светлана Анатольевна

**ПАТОЛОГИЧЕСКАЯ ФИЗИОЛОГИЯ  
СИСТЕМЫ КРОВИ**

**PATHOLOGICAL PHYSIOLOGY  
OF THE BLOOD SYSTEM**

Учебно-методическое пособие на английском языке

Ответственный за выпуск Ф. И. Висмонт  
В авторской редакции  
Компьютерный набор С. А. Жадан  
Компьютерная верстка Н. М. Федорцовой

Подписано в печать 29.04.10. Формат 60×84/16. Бумага писчая «КюмЛюкс».

Печать офсетная. Гарнитура «Times».

Усл. печ. л. 3,72. Уч.-изд. л. 3,91. Тираж 20 экз. Заказ 351.

Издатель и полиграфическое исполнение:  
учреждение образования «Белорусский государственный медицинский университет».  
ЛИ № 02330/0494330 от 16.03.2009.  
ЛП № 02330/0150484 от 25.02.2009.  
Ул. Ленинградская, 6, 220006, Минск.