**A N E M I A**

Anemias are characterized by a decrease in hemoglobin concentration and hematocrit level per unit volume of blood. Oxygen-carrying function of the blood decreases during anemia, that causes hypoxia. According to the WHO, regardless of age, gender, geography, the patient's hemoglobin level is less than 110 g/l, it is considered as anemia. In some types of anemia, the number of erythrocytes in the peripheral blood may not change or even increase (for example, in the minor form of thalassemia, iron deficiency anemia, the number of erythrocytes may sometimes be normal or slightly higher). Therefore, the main hallmark of anemia is the *concentration of hemoglobin* and the *level of hematocrit* (the ratio of red blood cells to the total volume of blood) is below the norm.

There are different types of anemia. Clinically, all of them are accompanied with the signs of *anemic syndrome*, and its manifestation depends on the degree of anemia, period of duration, the patient's age, concomitant diseases, and the compensatory capabilities of organism. General weakness, lack of appetite, physical and mental fatigue, dispnea, dizziness, tinnitus, and flashing "spots" in the eyes are typical signs of anemic syndrome. In severe cases, unconsciousness and coma may develop. During the examination, pallor of the skin and visible mucous membranes, tachycardia, hypotension, and expansion of the heart borders are determined. In auscultation, weakening of heart tones, systolic murmur is detected. Typical ECG changes are depression of the ST segment, flattened T wave, disturbance of heart rhythm. Ciliary arrhythmias can be detected in old aged patients.

Sometimes anemia appears not as an independent disease, but as a manifestation of another disease. In clinical practice, anemic syndrome is observed in the pathology of the liver, kidneys, chronic infectious diseases and malignant tumors. Therefore, not only the type and degree of anemia, but also the cause of anemia should be indicated in the final clinical diagnosis.

The type of anemia is mainly determined by the erythrocyte indices in the general blood analysis, changes in the blood smear, bone marrow regeneration capacity (in accordance with the change in the amount of reticulocytes in the peripheral blood). Therefore, when anemia is diagnosed, the results of the *blood analysis*, investigation of *peripheral blood smear*, which allows to determine the morphological changes, is necessary. Then, a *biochemical*  *examination of the blood* (also, immune enzymatic analysis) is performed. In cases of diagnostic doubt, a *bone marrow puncture* is performed and the cell composition of the *myelogram* is studied.

**Blood Analysis**

Venous, and sometimes capillary blood is taken for blood analysis. Venous blood is more preferred. In cases, where it is difficult to take venous blood, for examples, extensive burns, excessive obesity, tendency to venous thrombosis, newborns, etc. capillary blood for analysis is used. To obtain venous blood, the cubital vein is punctured (it can be any vein). Blood (mixed with anticoagulant) can be stored at 4°C for 24 hours. During this period, there are no significant changes in the number and morphology of cells. However, hematological studies are recommended as soon as possible, because pathological cells are often unstable. Soft tissues (the inner surface of the finger and, the earlobe or the heel in children) are punctured using sterile, disposable lancets (scarifies) to obtain capillary blood. Collected blood is mixed with anticoagulant. EDTA, sodium citrate or heparin are used as anticoagulants for hematological studies. General analysis of blood is carried out using automatic heme-analyzers.

Currently, automatic hematology analyzers are an important component of a modern clinical diagnostic laboratory (CDL). The electronic counter of the auto-analyzer can determine not only the number of cells, but also a number of other hematological parameters. The electronic counter analyzes a large number of blood samples quickly, minimizing the technical errors usually associated with manual counting. Modern hematological analyzers allow to perform a fully differentiated count of erythrocytes, to determine the number of reticulocytes by evaluating erythrocyte and platelet indices, their degree of maturity. Despite these advantages, the visual morphological assessment of blood cells has not lost its relevance. Therefore, in all doubtful cases of the pathology of the blood system, or if any abnormality is detected when examining a sample in a hematological analyzer, a morphological examination of the blood smear must be carried out.

**The main indicators of general blood analysis determined in automatic analyzers and their abbreviations:**

|  |  |  |
| --- | --- | --- |
| Abbreviation | Index | Norma |
| WBC | White Blood Cell | 4,0-9,0x109/l |
| RBC | Red Blood Cell | F: 4,8±0,6х1012/l  M:5,4±0,8х1012/l |
| Hb | Hemoglobin | F: 140± 20 g/l  M: 160± 20 g/l |
| Hct | Hematocrit | F: 42 ± 5%  M:47 ± 5% |
| MCV | Mean Corpuscular Volume | 87 ± 5 fl |
| MCH | Mean Corpuscular Hemoglobin | 29 ± 2pg |
| MCHC | Mean Corpuscular Hemoglobin Concentration | 34 ± 2 % |
| RDW | Red cell Distribution Width | 11,5-14,5 % |
| Plt | Platelets | 180,0-320,0x109/l |
| MPV | Mean Platelet Volume | 8-12 fl |
| PDW | Platelet Distribution Width | 11,5-15,5 % |
| Pct | Thrombocrit | 0,15-0,50 % |
| LY % | Lymphocytes, % | 19-37 % |
| LY# | Lymphocytes, # | 1,20-3,00х109/l |
| MO% | Monocytes, % | 3-11% |
| MO# | Monocytes, # | 0,09-0,60х109/l |
| NE% | Neutrophils, % | 48-78 % |
| NE# | Neutrophils, # | 2,04-5,8х109/l |
| EO% | Eosinophils, % | 0,5-5 % |
| EO# | Eosinophils, # | 0,02-0,30х109/l |
| BA% | Basophils, % | 0-1 % |
| BA# | Basophils, # | 0-0,065х109/l |
| ESR | Erythrocyte Sedimentation Rate | F: 2-15 mm/hour  M: 1-10 mm/hour |

#- absolute number of cells

The indicators given in the above table and the information about their meanings are widely explained in the pathological physiology course. Differentiation of anemias sometimes the is based on the change of these indicators.

**Differentiation of Anemia depending of МCV:**

|  |  |  |
| --- | --- | --- |
| МCV < 80 fl  Microcytic anemia | MCV 80–100 fl  Normocytic anemia | MCV ˃100 fl  Macrocytic anemia |
| Iron deficiency anemia  Thalassemia  Anemia of chronic diseases  Sideroblastic (sideroachrestic) anemia | Acute posthemorrhagic anemia  Aplastic anemia  Hemolytic anemias  Anemia in chronic diseases  Hereditary dyserythropoietic anemias | Megaloblastic anemia  Aplastic anemia (hereditary forms)  Hemolytic anemias accompanied by chronic hemolysis  Myelodysplastic syndrome |

Depending on MCH and MCHC, anemias are divided into *normochromic, hypochromic and hyperchromic types*. MHC decreases in iron deficiency anemia, hemoglobinopathies, sideroacrestic anemias; its increase is found in macrocytic and especially megaloblastic anemias.

**Types of anemia depending of MHC and MCHC**

|  |  |  |
| --- | --- | --- |
| Hypochromic | Normochromic | Hyperchromic |
| *МСН* – ˂ 24 pg | *МСН* – 24–34 pg | *МСН* – ˃ 34 pg |
| *МСНС* – ˂ 30 g/l | *МСНС* – 30–38 g/l | *МСНС* – ˃38 g/l |

***The main characteristics of erythrocytes in peripheral blood smear***

Studying the morphological characteristics of erythrocytes during anemia provides the physician with important information. Therefore, microscopic examination of blood smear is important for the diagnosis and differentiation of anemia.

During the microscopic examination of the peripheral blood smear, the following parameters are mainly evaluated:

*Size of erythrocytes.* Normally, erythrocytes are about the same size as lymphocytes (6.5-8 µm or 80-100 fl). Detection of erythrocytes of different sizes in peripheral blood smear is called *anisocytosis*. RDW (red blood cell distribution width, normally 11.5-14.5%) - is an indicator of erythrocyte anisocytosis, allows to characterize the volume variability of erythrocytes. An increase in RDW characterizes a high degree of heterogeneity in the size of erythrocytes. The predominance of erythrocytes with a diameter of less than 6.5 microns (smaller than a lymphocyte) in a blood smear is called *microcytosis*, and erythrocytes with a diameter of more than 8 microns are called *macrocytosis*. Erythrocytes with a diameter of more than 10-12 microns are *megalocytes*.

Anisocytosis is characteristic of most anemias of various origins. The nature of anisocytosis allows the physician to narrow the range of diagnostic researches. For example, microcytosis is characteristic of iron-deficiency anemia, thalassemias, and macrocytosis is characteristic of megaloblastic anemias.

Physiological anisocytosis (macrocytosis) occurs in newborns in the first two weeks of life, usually disappears after two months.

*The shape of erythrocytes.* Poikilocytosis is a change in the shape of red blood cells. The fact of poikilocytosis, as in anisocytosis, is noted when poikilocytes make up about 25% or more of erythrocytes. At the same time, it is necessary to indicate which form of erythrocytes is represented by poikilocytosis, because some variants are characteristic of a certain pathology (for example, microspherocytes - in Minkowski-Shoffar disease, sickle cells - in sickle cell anemia).

|  |  |
| --- | --- |
| Poikilocytes | Anemia |
| Microspherocytes | Hereditary microspherocytosis, immune hemolytic anemias, G-6-PD enzymopathy of erythrocytes, microangiopathic hemolytic anemia |
| Target cells | Thalassemia, hemoglobinopathies, liver diseases, iron deficiency, post-splenectomy condition |
| Ovalocytes (ellipsocytes) | Hereditary ovalocytosis, megaloblastic anemia, iron deficiency, thalassemia, anemia during leukemia |
| Stomatocytes | Hereditary stomatocytosis - hemolytic anemia |
| Drepanocytes | Sickle cell anemia |
| Toothed erythrocytes | Uremia |
| Acanthocytes | Hereditary acanthocytosis (a form of hereditary hemolytic anemia), severe forms of liver diseases |
| Anulocytes | Hypochromic anemia |
| Shizocytes | DİC, uremia, hemolytic-uremic syndrome, hemolysis of erythrocytes by a mechanical and toxic factors |
| Droplet-like erythrocytes | Myeloproliferative diseases, myelofibrosis, thalassemia, iron deficiency, megaloblastic anemia |
| Bited erythrocytes | G-6-PD enzymopathy of erythrocytes |

*Characteristics of erythrocyte staining* - in the blood smear erythrocytes are stained pink with acidic dyes. The central area (about 1/3) of these cells are paler (colorless), and the edges are more intensively stained.

*Hypochromia,* is associated with pale stained erythrocytes and characterized by a wider central pale area. Hypochromia is characteristic of iron deficiency, sideroblastic anemia, thalassemia.

*Hyperchromia,* is associated with intense staining of erythrocytes and the absence of a central pale part are determined. It should be noted that the hyperchromia of erythrocytes is not related to the increase in the amount of hemoglobin inside of them (because the "saturation" of erythrocytes with hemoglobin cannot exceed a certain limit) it is related to the change in the shape of these erythrocytes. For example, in microspherocytic anemia, erythrocytes become hyperchromic because they lose their discoid shape and become spherical.

*Anisochromia* is accompanied by the presence of erythrocytes with different color intensity in the peripheral blood smear.

*Polychromatophilia* is a condition in which erythrocytes are stained with both acidic and basic dyes, obtaining shades from gray-pink to blue-violet. This is due to the presence of an alkaline substance in the cytoplasm of young erythroid cells, as well as an acidic substance in erythrocytes. The detection of polychromatophilia in peripheral blood smears stained with Romanowski-Giemza indicates an increased number of reticulocytes. It should be noted that special staining methods are needed to count the number of reticulocytes.

*Intracellular derivatives* - can usually be detected in erythrocytes in pathological cases:

|  |  |
| --- | --- |
| *Jolly bodies* | Nuclear remnants are found in anemia caused by vitamin B12 and folic acid deficiency, as well as after splenectomy |
| *Kebot rings* | Remnants of the nuclear membrane are found in B12 and folic acid deficiency anemia, polycythemia and heavy metal salt poisoning |
| *Heinz bodies* | Denatured Hb precipitates are detected during hemolytic crises in patients with hereditary deficiency of glucose-6-phosphate dehydrogenase |
| *Basophilic granulation* | Occurs during lead or heavy metal poisoning, thalassemia, alcohol intoxication, cytotoxic effect of drugs, severe anemia |
| *Siderosis (iron granules)* | An increase in their number is observed in hemolytic, sideroblastic anemia, splenectomy, lead poisoning, thalassemia |

*Reticulocytes* are young erythrocytes that contain remnants of RNA. Normally, the amount of reticulocytes in the blood is 1-2%. *Reticulocytosis-* an increase in the number of reticulocytes in the peripheral blood is a sign of the activation of normoblastic hematopoiesis and indicates a high regenerative capacity of the bone marrow. The highest degree of reticulocytosis is observed in hemolytic anemia. Reticulocytopenia is an indicator of inhibition of erythropoiesis and replacement of normoblastic hematopoiesis with megaloblastic (in case of megaloblastic anemia), as well as characteristic of aplastic states of hematopoiesis.

**Differentiation of anemias according to the regenerative properties of bone marrow.**

|  |  |  |
| --- | --- | --- |
| Hyperregenerative  Reticulocytes ˃5-10% | Regenerative  Reticulocytes ˃ 3% | Hypo-, aregenerative  Reticulocytes ˂1% |
| Acute posthemorrhagic anemia  Hemolytic anemia (mainly acquired)  Iron deficiency with bleeding  Reticulocyte crisis during treatment of B12 and folate deficiency anemia | Iron deficiency anemia (early stage  Anemia of chronic diseases (early stage)  Thalassemia | Aplastic anemia  Megaloblastic anemia  Sideroblastic anemia  Myelodysplastic syndromes |

The study of peripheral blood smear plays a very important role in the interpretation and monitoring of changes in the morphology of erythrocytes.

In addition to general blood analysis and peripheral blood smear examination, also, *biochemical blood analysis* and, if necessary, *bone marrow biopsy examination* are carried out. Biochemical analysis of blood mainly allows to study the amount of serum iron, the level of serum ferritin, which reflects the iron reserve in the tissues, as well as the level of transferrin, which transports iron in the serum. The dynamic changes in the amount of such substances as vitamins, their metabolites, as well as erythropoietin, haptoglobulin, bilirubin, etc. are of diagnostic importance. Beside of these, pathological types of hemoglobin (HbA2, HbH, HbF, HbS, etc.) are detected by the electrophoresis method in hereditary types of anemia. The presence of antibodies against erythrocytes in blood is studied by means of serological examinations. Thus, the mentioned examinations are of decisive importance in determining the diagnosis of anemia in most cases.

**Pathogenetic Classification of Anemias**

|  |
| --- |
| I. ANEMIA DUE TO HEMORRHAGİA  • Acute post-hemorrhagic anemia  • Chronic posthemorrhagic anemia |
| II. ANEMIA DUE TO DISORDER OF ERYTHROPOESIS AND HEMOGLOBIN SYNTHESIS  A. Bone Marrow Insufficiency  •Aplastic anemia (total)  - Congenital (Fanconi anemia, Estrana-Dameshek anemia)  - Acquired (under the influence of infectious-toxic factors)  • Partial red cell aplasia  - Congenital (Blackfen-Diamond anemia)  - Acquired (Parvovirus B19 infection)    B. Pathology of Erythrocyte Maturation.  1. Anomaly of hemoglobin synthesis  • Iron deficiency anemia  • Sideroblastic (Sideroachrestic) anemia  - Congenital  - Acquired  • Anemia of chronic diseases  2. Anomaly of DNA and RNA synthesis  • Vitamin B12 deficiency  • Folic acid deficiency  • Hereditary disorders of folate metabolism |
| III. ANEMIAS ASSOCIATED WITH HEMOLYSIS OF ERYROCYTES  A. Hereditary Hemolytic Anemias  • Genetic defects of the erythrocyte membrane –membranopathies (Minkowski-Schoffard anemia, congenital ellipsocytosis (ovalocytosis), congenital stomatocytosis)  • Defects in enzyme systems of erythrocytes – enzymopathies (glucose-6-phosphate dehydrogenase deficiency)  • Structural defects of hemoglobin – hemoglobinopathies ( sickle cell anemia, thalassemias)  B. Acquired Hemolytic Anemias  • Immune hemolytic anemias  - Isoimmune (incompatibility due to ABO or Rh factor  - Heteroimmune (infectious, toxic, medicinal)  - Autoimmune (associated with warm antibodies, cold agglutinins, cold hemolysins)  • Mechanical Hemolytic Anemias  - Thrombotic microangiopathic hemolytic anemias  - Damage to the valves of the heart and large vessels  - Hemoglobinuria during march and anemia of athletes  • Paroxysmal nocturnal hemoglobinuria  •Drug-related hemolytic anemia  • Anemias associated with pathological activation of complement system  • Hemolytic anemia caused by toxins  • Hemolytic anemia caused by destruction of red blood cells by parasites  • Hypersplenism |

Some types of anemia and their clinical laboratory diagnosis are described below.

**Acute Posthemorrhagic Anemia**

Acute posthemorrhagic anemia develops as a result of acute bleeding. Regardless of the etiological factor, during acute bleeding, the activation of mechanisms aimed to restore the volume of circulating blood is reflected in the laboratory indicators. Changes in the blood develop in stages - in a certain sequence.

*In the first stage* (in first 8-12 hours), spasm of peripheral vessels, decrease in the volume of the vascular lumen and the mobilisation of blood from the depots to the general circulation are observed. At this stage after blood loss, the amount of hemoglobin and erythrocytes approaches the previous level, as a result, despite the fact that the absolute amount of erythrocytes decreases, indicators reflecting the true degree of anemia are not revealed. Transient thrombocytopenia may develop immediately after bleeding, but after a few hours, *thrombocytosis, leukocytosis,* and  *shift to the left in the leukocyte count* are observed.

*In the second stage (1-2 days after hemorrhage),* hemodilution develops - tissue fluid enters the bloodstream, as a result of which the volume of circulating plasma is restored. As the number of erythrocytes and the amount of hemoglobin decrease gradually and equally, there is no decrease in color index. At this stage initially *normochromic-normocytic* anemia develops.

*In the third stage* ( 4-5 days after hemorrhage) acute *reticulocytosis* develops, such activation of bone marrow regenerative capacity reaches its maximum in 7-10 days, leukocytosis is 12-20x109/l, nuclear shift to the left is observed. As a result of reticulocytosis, the picture of the blood becomes polychromatophilic-macrocytic, MCV increases. At this time, anemia can be macrocytic normochromic. Reticulocytosis and an increase in MCV can lead to a false diagnosis of hemolytic anemia. Anemia that develops during acute bleeding is *normocytic and normochromic*.

After the stopping of hemorrhage, the reticulocyte count returns to normal in 2-3 weeks. Otherwise, continued reticulocytosis indicates continued bleeding. Acute bleeding usually does not cause anemia if the bleeding is prevented in time and the body has sufficient iron stores.

The minimum blood loss that threatens human life is 500 ml, acute blood loss of 1/4 of the total volume of blood can cause shock, loss of half of the total volume of blood results in death.

Chronic bleeding leads to depletion of iron reserves in the body and disruption of hemoglobin biosynthesis. These lead to the development of hypochromic- microcytic anemia.

**Iron Deficiency Anemia**

**↓Iron→↓Heme→↓Hemoglobin→microcytic-hypochromic anemia**

Iron deficiency anemia (IDA) ranks first among the world's population. According to the WHO, about 2 billion people on Earth suffer from IDA. In addition to iron deficiency anemia, latent (hidden) iron deficiency is also distinguished, the prevalence of latent iron deficiency reaches 3.6 billion people.

The total amount of iron in the body is 2g in women and 6g in men. 60-65% of it is iron contained in hemoglobin, and the rest (30-40%) is iron contained in *ferritin* and *hemosiderin*, which are stored in the liver, spleen, bone marrow and muscles. Ferritin is an apoferritin-protein complex, contains 20% iron, water-soluble, and easily used for erythropoietic needs. Hemosiderin is a water-insoluble protein, contains more (25-30%) iron, a stable part of iron reserves. Since ferritin enters the plasma mainly from the reserve pool, the level of iron in the plasma indicates the state of iron reserves in the body. Serum ferritin is always lower than normal in iron deficiency, and higher in case of iron overload.

In the plasma iron is transported by glycoprotein – *transferrin* that synthesized in the liver. In healthy people, transferrin is ~35% saturated with iron. The main function of transferrin is to deliver iron to all cells, most importantly to erythroid cells that require iron for hemoglobin synthesis. The tissue fund of iron is 1%, which is represented in the form of iron-containing enzymes (cytochromes, catalase) and myoglobin.

Iron enters the body in both *heme* (in meat) and *non-heme* (in vegetable) forms. Iron absorption occurs in the enterocytes of the duodenum. *Ferroportin*  plays a role in the transfer of iron from enterocytes to the blood. The activity of ferroportin is regulated by *hepcidin*, which is synthesized by the liver. Hepcidin has the ability to inactivate ferroportin. When the body is overloaded with iron, the level of hepcidin also increases and the absorption of iron is limited. On the contrary, when iron reserves decrease, the synthesis of hepcidin also decreases, which facilitates the absorption of iron. By inhibiting ferroportin, hepcidin not only limits iron absorption from intestinal enterocytes, but also reduces iron release from macrophages. Macrophages are the main source of iron used by the hemoglobin-synthesizing erythroid cells of the bone marrow. This mechanism plays an important role in the pathogenesis of anemia during chronic diseases.

Iron is delivered to all tissues in the form of transferrin in the blood. Usually 1/3 of transferrin is enriched with iron. This is called serum iron. Normal serum iron content is about 1000 μg/L. The part of transferrin that is not bound to iron is called latent iron binding capacity (LIBC) of serum. The maximum amount of iron that transferrin can combine for complete saturation (enrichment) with iron is called total iron binding capacity - TIBC (normal TIBC is 30-85 μmol/L) of serum. Thus, TIBC = Serum Iron+LIBC. The ratio of serum iron to TIBC indicates the percentage of transferrin saturation with iron (norm 35%).

**To determine the level of iron, the following laboratory indicators are used:**

|  |  |  |
| --- | --- | --- |
|  | | |
| Serum Iron | 1000 μg/l | indicates the amount of iron in the blood |
| Serum ferritin | 12-32μM/l | reflects the level of ferritin in the liver and macrophages |
| (TIBC) total iron binding capacity | 30-85μMl/l | indicates the amount of transferrin molecules in the blood |
| (LIBC) latent iron binding capacity |  | the amount of the part of transferrin that does not combine with iron |
| Transferrin % saturation | 33-35% | saturation degree |

**Soluble transferrin receptors (sTFR)** - recently this indicator is used to evaluate the level of iron in the body. Transferrin receptor is a transmembrane protein located on the membrane surface of cells. The stable peptide released from this receptor is soluble in plasma. This is called the soluble receptor of transferrin, the determination of its concentration in the serum is considered a factor that more accurately shows the level of iron in the body. During iron deficiency sTFR increases, and vice versa, in iron overload it decreases.

**Etiopathogenesis.** The causes of iron deficiency are: (1) lack of iron in food; (2) malabsorption; (3) increased need for iron; (4) chronic blood loss (in most cases). Women of childbearing age, children, and teenagers in the period of rapid growth are most at risk of IDA.

Dietary iron deficiency is rare in developed countries. ~65% of dietary iron is in meat and is in the form of heme, which is easily absorbed. On the contrary, in developing countries, the source of iron is mainly in plant-based foods - poorly absorbed inorganic form. Iron deficiency in food is noted in special groups of the population:

• breastfed babies due to the very low amount of iron in breast milk

• poor people who are undernourished for economic reasons

• older people who eat less meat due to low income or dental problems

*Malabsorption syndrome* develops during sprue, steatorrhea and chronic diarrhea, etc. Ascorbic acid, citric acid, amino acids and sugars in food increase the absorption of iron in non-heme form, whereas tannic acid salts in tea, carbonates, oxalates and phosphates slow down this process.

The increased need for iron is an important cause of iron deficiency in children and adolescents, as well as in women during pregnancy and premenopause. Women from poor families with many children have a very high risk of developing iron deficiency.

Chronic blood loss is one of the most common causes of iron deficiency in western countries. Gastrointestinal bleeding, uterine bleeding, bleeding during tumors, blood loss during donation, iron reserves are depleted during hemodialysis. Careful clinical examination of a patient with unexplained iron deficiency anemia can sometimes reveal hidden bleeding or tumor, thereby saving the patient's life.

In very rare cases, the cause of iron deficiency is a violation of its transportation as a result of a congenital defect in the synthesis of transferrin (atransferrinemia).

The clinical picture of iron deficiency anemia is characterized by symptoms common to all anemias – called *anemic syndrome*. In case of long-term severe iron deficiency, clinical manifestations related to the lack of iron-containing enzymes and proteins. *Koilonychia* (indentation of the nail surface), *alopecia* (focal baldness), atrophy of the mucous membrane of the oral cavity and other parts of the digestive tract, *picasism* (parorexias such as eating of raw meat, clay, raw dough , and liking the smell of substances such as gasoline, varnish, acetone, etc.), *Plummer-Vinson syndrome* (a triad consisting of microcytic anemia - atrophic glossitis - muscular degeneration of the esophagus) is observed.

In case of iron deficiency, there is an imbalance between the need for iron and its intake. At this time, first of all, the amount of ferritin decreases, then the amount of iron in the serum, next - the iron of heme-containing enzymes, and finally, the iron necessary for the synthesis of hemoglobin decreases. The decrease in iron is accompanied by a decrease in the level of hepcidin in the serum.

It should be noted that determining the patient's iron deficiency is only the beginning of the diagnostic search, and the main reason for the development of the disease must be revealed.

**Laboratory diagnosis of IDA is carried out in stages.** First, general blood analysis reveals hypochromic anemia. The hypochromic nature of anemia determines the direction of the diagnostic search to first suspect IDA and confirm iron deficiency in the body and determine its cause. Thus, IDA is always hypochromic, but not all hypochromic anemias are associated with iron deficiency.

**Changes observed in the general analysis of blood during IDA:**

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| --- |
| * decrease in hemoglobin level Нb <98 g/l * decrease in hematocrit * moderate reduction of erythrocytes (reduction of erythrocytes more than <2x1012/l is not characteristic of IDA) * decrease of erythrocyte indices: MCV, MCH, MCHC * microcytic-anisocytosis, increased RDW * reticulocytes are normal or slightly increased, their number decreases as the disease progresses * WBCs are normal but there is a tendency towards leukopenia due to neutropenia (~ 10% of IDA) * platelets are normal, but mild thrombocytosis may occur with the development of DDA against the background of chronic blood loss * ESR is normal or increased (with a significant decrease in the number of erythrocytes) |

In the blood smear, hypochromia, widening of the central-pale part of erythrocytes, annulocytes (ring-shaped erythrocytes with an empty inner area), target-like erythrocytes, microcytes, ovalocytes are visible.



Microcytosis, hypochromia in iron deficiency anemia

Biochemical blood analysis is important to confirm the fact of iron deficiency in the body. The following changes are observed in the biochemical analysis of blood:

|  |
| --- |
| * decrease in serum iron level * decrease in serum ferritin level * increase of TIBC and LIBC * decrease in iron saturation % of transferrin * the level of sTFR in the serum increases * the level of hepcidin in the serum decreases |

An important indicator in determining of iron deficiency is serum ferritin, an indicator that reflects the amount of iron in the depots of the body. At the same time, it should be taken into account that ferritin is one of the acute phase proteins and its level can increase against the background of inflammatory processes.

Bone marrow examination (myelogram) is not mandatory in IDA. This examination is performed when the therapy with iron preparations does not give a positive result, in case of persistent thrombocytopenia and leukopenia.

During IDA, additional examinations are also performed to clarify the cause of iron deficiency. These examinations are selected individually, depending on the patient's age, sex, accompanying diseases. For example, examination of the gastrointestinal tract to identify diseases that cause bleeding, assessment of the amount of blood loss during menstruation in women, determination of blood in feces, fibrogastroduodenoscopy, colonoscopy, identification of parasitic infestations, urinalysis, etc. such examinations are conducted.

Laboratory tests are important not only for the diagnosis of IDA, but also for monitoring the effectiveness of treatment. Treatment is carried out by taking iron preparations. A sign of successful treatment with iron preparations is the complete normalization of the serum ferritin level.

**Anemia of Chronic Disease**

**↓Available Iron for Hemepoiesis → ↓Heme → ↓Hemoglobin → microcytic-hypochromic anemia**

Anemia of chronic diseases (ACD) is a fairly common pathology and ranks second among all anemias (after iron deficiency anemia) in terms of frequency of development. ACD develops in infectious-inflammatory diseases (osteomyelitis, bacterial endocarditis, lung abscess, sepsis, tuberculosis), immune and autoimmune pathologies (allergies of various etiologies, rheumatoid arthritis, enteritis), neoplasias (lung and breast carcinomas, Hodgkin's lymphoma).

The mechanism of development of *hypochromic anemia* during these diseases is the lack of iron available for hemoglobin synthesis as a result of redistribution of iron in the body and violation of reuse (reutilization) of iron in depots. All this is due to an increase in the level of *hepcidin* in the blood. In chronic diseases, inflammatory mediators (especially IL-6) increase the synthesis of acute phase proteins, including hepcidin, from the liver.

Hepcidin: 1) sequesters iron in storages sites 2) limits the transfer of iron from macrophages to erythroid cells, and 3) inhibits the synthesis of erythropoietin. As a result, erythroid precursor cells are exposed to iron "starvation" in conditions of iron excess in depots. Sharply decreasing of the level of erythropoietin causes a delay in proliferation of these cells that is not typical for other hypochromic anemias. Activation of the macrophages, the storage of iron in the activated macrophages, and the process of reutilization of iron are disturbed in the above-mentioned diseases.

What is the reason for sequestration of iron during inflammation? It is believed that since iron is needed for the pathogenic activities of many infectious agents, during these diseases, the reduction of iron in the plasma and retention in stores is an adaptation reaction to increase the body's resistance. It should also be noted that hepcidin is structurally similar to defensins (a natural peptide with antibacterial activity).

**Typical changes in ACD:**

|  |
| --- |
| * decrease in the hemoglobin level (<80 g/l) is noted * increasing of the level of serum ferritin, which indicates an increase in the amount of iron in the depots * serum iron level decreases moderately * TDBQ decreases, which indicates absence of serum Fe-starvation * transferrin saturation % decreases * decrease in the level of erythropoietin in the blood |

Anemia in chronic diseases is usually mild, the symptoms of the underlying disease predominate. Erythrocytes can be microcytic hypochromic as in iron deficiency anemia, but sometimes they are *normocytic, normochromic*. Excess iron stores in bone marrow macrophages, elevated serum ferritin levels, and decreased serum TIBC rule out the iron deficiency anemia. Only successful treatment of the underlying disease allows to eliminate anemia. Treatment with iron preparations is ineffective, treatment with erythropoietin gives a positive result.

**Sideroblastic Anemia (SBA)**

**↓Protoporphyrin→ ↓Heme→ ↓Hemoglobin → microcytic-hypochromic anemia**

Sideroblastic (or siderochrestic) anemias relate to *hypochromic-microcytic- hyporegenerative* type of anemia. Despite normal or excess iron in the mitochondria of erythroblasts, it occurs as a result of a violation of the intracellular utilization of iron for the synthesis of hemoglobin. Thus, due to hereditary and acquired reasons, the activity of enzymes involved in the synthesis of porphyrin and heme is disturbed. A lot of iron accumulates in tissues, but iron cannot be used for the synthesis of porphyrins – an intermediate product of heme synthesis. As a result, the number of sideroblasts increases in the bone marrow. Sideroblasts are ring-shaped cells distinguished by the characteristic annular arrangement of iron granules around their nucleus.

There are hereditary and acquired forms of sideroblastic anemia. Hereditary forms are rare, occurring mainly in men. Acquired forms occur against the background of lead poisoning, sometimes vitamin B6 deficiency, and ethanol intoxication.

Disruption of hemoglobin synthesis leads to a decrease in the average amount of hemoglobin in the erythrocyte, and a population of *hypochromic microcytes* appears. Erythroid hyperplasia of the bone marrow occurs, ring-shaped sideroblasts are observed in the smear.

**Changes in the blood analysis are observed in sideroblastic anemia:**

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| * increasing of the serum ferritin level * significantly increasing of the serum iron * transferrin saturation % is high * the amount of reticulocytes decreases - hyporegenerative anemia develops * along with hypochromic microcytic cells, normocytes and macrocytes are observed |

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| The presence of normocytes-macrocytes along with hypochromic-microcytic cells in the smear is a diagnostic sign of sideroblastic anemia. |

Iron accumulates in various organs and causes their dysfunctions in SBA. Accumulation of iron in the liver can lead to cirrhosis, in the pancreas – to diabetes, in the adrenal glands - to adrenal insufficiency, and in the myocardium - to the heart failure.

Hypochromia that is a common sign characteristic for IDA, ACD, SBA and also for thalassemia, is already known from a general blood analysis. However, the results of the biochemical analysis of blood are necessary to clarify which one of hypochromic anemias have developed in the patient.

**Differentiation of hypochromic anemias**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Index | IDA | ACD | SBA | Thalassemia |
| Serum Iron | Decreases | Decreases | Increases | Increases |
| TIBC | Increases | Decreases | Decreases | Decreases |
| Serum Ferritin | Decreases | Increases | Increases | Increases |
| TFR-saturation % | Decreases | Decreases | Increases | Increases |
| CRP | Normal | Increases | Normal | Normal |
| sTFR | Increases | Normal/ Decreases | Decreases | Decreases |
| Erythrocytes morphology | Mikrositoz | Normal/microcytois | Dimorphism | Target cells |

**Megaloblastic Anemia**

**↓Vitamin B12 → ↓FH4 → ↓DNA synthesis → megaloblastosis → macrocytic-hyperchromic anemia**

Megaloblastic anemias include a group of acquired and hereditary anemias, which occur when normoblastic hematopoiesis in the bone marrow is replaced by megaloblastic type. The frequency of these anemias is 9-10% of all anemias. Types of megaloblastic anemias include *vitamin B12 deficiency anemia, folic acid deficiency anemia,* and *pernicious* (malignant) *anemia*.

Vitamin B12 and folic acid are coenzymes necessary for the synthesis of thymidine, a component of DNA. Deficiency of vitamin B12 and folic acid or disruption of their metabolism limits DNA synthesis. As a result, the maturation of the nucleus and cell division are delayed. While nuclear division and maturation are delayed, cytoplasmic maturation and hemoglobin synthesis proceed at a normal rate, asynchronous nuclear-cytoplasmic development occurs. Since DNA synthesis is disrupted not only in erythrocytes, but in all cells, the maturation of progenitor cells of granulocytes is also disrupted, and they turn into giant metamyelocytes and matured neutrophills. At the same time, megakaryocytes with unusually large and multilobular nuclei appear.

The main causes of vitamin B12 deficiency are its lack in food (mainly observed in vegetarians), impaired absorption of vitamin B12 from the intestine (autoimmune pernicious anemia), helminth invasion, tumor or resection of the small intestine, chronic enteritis, malabsorption syndrome, etc. In rare cases, the delivery of vitamin B12 to the bone marrow and its depots is impaired as a result of a hereditary deficiency of transcobalamin.

Serum levels of *homocysteine* and *methylmalonic acid* are elevated in vitamin B12 deficiency. This is related to the metabolism of vitamin B12. Thus, in the metabolism of vitamin B12, two coenzymes are formed - methylcobalamin and 5-deoxyadenosylcobalamin. Methylcobalamin is an important cofactor for the conversion of homocysteine to methionine. As a result of this reaction, tetrahydrofolic acid (FH4), the main active form of folic acid necessary for DNA synthesis, is formed. As a result of methylcobalamin deficiency, FH4 formation and DNA synthesis are impaired, mitotic division of cells is delayed, and the level of homocysteine in the plasma increases (this is a risk factor for atherosclerosis and thrombosis). Another metabolite, 5-deoxyadenosylcobalamin deficiency, increases the level of methylmalonic acid, which accumulates in nerve cells and causes their fatty degeneration and demyelination.

Changes in peripheral blood are characteristic of all megaloblastic anemias. The most characteristic feature is the presence of *macrocytic oval-shaped erythrocytes - macroovalocytes.* These cells are larger than normal and lack the central pale area that is characteristic of normal erythrocytes. *Anisocytosis* and *poikilocytosis* are characteristic features. The cells are rich in hemoglobin and are *hyperchromic*, but the average concentration of hemoglobin in erythrocytes is low. In some cases, nucleated erythrocytes are also observed in the blood. Neutrophils are also larger than normal (macropolymorphonuclear cells), have 5 or more (normally 3-4) nuclear segments (*hypersegmentation)*.

If there is a deficiency of vitamin B12 in the body, megaloblastic changes are detected in the bone marrow within 1-2 years, the average size of erythrocytes (MCV) increases. Anemia in peripheral blood develops after 6-18 months.



*Macrocytic, hyperchromic* anemia develops during B12 deficiency. Examination of the smear shows *polychromatophilia, Cabot rings, Jolly bodies,* and *basophilic punctuation* in erythrocytes. During B12 deficiency, the number of *reticulocytes is reduced* against the background of macrocytic, hyperchromic anemia.

C**hanges are observed in vitamin B12 deficiency anemia**

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| * the number of erythrocytes decreases sharply (1.0 - 1.5x1012/l) * MCV >100 fl * MCH > 32 pg * giant hypersegmented neutrophils * the number of reticulocytes decreases * pancytopenia is observed * the level of homocysteine and methylmalonic acid increases * the level of vitamin B12 in the serum decreases * glossitis is characteristic * neurological changes are observed |

In most patients, the number of leukocytes (mainly neutrophils) decreases. The leukocyte formula shows shift to the right, and giant hypersegmented neutrophils. The number of eosinophils and monocytes decreases, relative lymphocytosis occurs. In about half of the patients, the number of platelets decreases significantly.

Biochemical analysis of blood shows that the level of vitamin B12 in the serum is below the norm (the norm for adults is 148 - 616 pmol/l).

In clinical practice, the diagnosis of vitamin B12 deficiency anemia is confirmed by an increase in reticulocytes (*reticulocyte crisis*) in response to the administration of minimal doses of vitamin B12 intramuscularly (2 μg/day).

**Folic Acid Deficiency Anemia**

**↓FH4→↓DNA synthesis → megaloblastosis → makrocytic - hyperchromic anemia**

Folic acid deficiency also results in megaloblastic anemia, as the vitamin B12 deficiency. Thus, deficiencies of vitamin B12 and folic acid are the direct cause of DNA synthesis disorders and megaloblastosis.

The main causes of folic acid deficiency are the decrease in its food intake (more in the poor or aged population, chronic alcoholics). An increasing of the demand for folic acid is observed in pregnancy and neonatal periods, in hemolytic anemia (due to hyperactive hematopoiesis), during malignant tumors. Its absorption is impaired during intestinal resection, in Crohn's disease, etc.

The richest sources of folic acid are greens, especially lettuce, spinach, asparagus and broccoli. Folic acid is sensitive to high temperature (95% of folic acid in food is destroyed in 5-10 minutes during boiling and frying).

Folic acid antagonists (methotrexate) inhibit its activity and cause FH4 (tetrahydrofolic acid) deficiency. When folic acid metabolism is disturbed, all rapidly proliferating cells are damaged, especially cells in the bone marrow and the gastrointestinal tract.

Megaloblastic anemia in cases of folic acid deficiency manifested by the similar sings as in vitamin B12 deficiency, so the diagnosis of folic acid deficiency can be made after determining a decrease in folate levels in serum or erythrocytes. As in vitamin B12 deficiency, *serum homocysteine levels are elevated* in folic acid deficiency, but *methylmalonate levels are normal* and *neurological changes do not develop*.

A positive hematological response during treatment is *reticulocytosis*, that occurs after taking of folic acid *per os*. It should be noted that treatment with folic acid also eliminates symptoms of vitamin B12 deficiency. However, folic acid supplementation does not prevent (or even exacerbate) the neurological symptoms of vitamin B12 deficiency. In this regard, it is necessary to rule out vitamin B12 deficiency before starting treatment with folic acid in megaloblastic anemia.

**Pernicious Anemia**

**↓↓↓IF→↓ vitamin B12 → ↓FH4→ ↓DNA synthesis → megaloblastosis → makrocytic-hyperchromic anemia**

Pernicious anemia (or Birmer's anemia) is an autoimmune disease resulting from the formation of antibodies against the parietal cells in the stomach or Casl's intrinsic factor (*IF-intrinsic factor*). This factor plays an important role in prosess of vitamin B12 absorbtion from intestine. In this form of megaloblastic anemia, autoimmune atrophic gastritis causes a deficiency of intrinsic factor, which in turn causes a deficiency of vitamin B12. The diagnosis of pernicious anemia is based on the following changes:

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| * antibodies against intrinsic factor or gastric parietal cells * symptoms of moderate and severe megaloblastosis * hypersegmented granulocytes against the background of leukopenia * low level of vitamin B12 in serum * increase in serum levels of homocysteine and methylmalonic acid |

In the laboratory diagnosis of these anemias, the determination of *antibodies against the intrinsic factor* is a great importance. Antibodies against intrinsic factor in serum are found in 50-70% of patients with pernicious anemia. *Antibodies against gastric parietal cells* are determined in about 90% of patients with atrophic gastritis and pernicious anemia.

The finding of the mentioned antibodies indicates that megaloblastic anemia is of an autoimmune nature. Other clinical laboratory indicators are the same as in vitamin B12 deficiency anemia.

*Macro- and megalocytosis, ovalocytosis, thrombocytopenia* and *leukopenia and pancytopenia* develop in the blood. Serum levels of *homocysteine* and *methylmalonic acid are elevated* in patients with pernicious anemia due to vitamin B12 deficiency. High levels of homocysteine increase the risks of atherosclerosis and thrombosis, while accumulation of methylmalonic acid causes demyelination of nerves.

People with pernicious anemia and atrophic or metaplastic changes in the gastric mucosa are at risk of developing gastric carcinoma.

A sharp increase in the number of reticulocytes and normalization of the hematocrit about 5 days after parenteral administration of vitamin B12 confirms the diagnosis. Anemia can be treated with high-dose parenteral administration of vitamin B12. At this time, peripheral neurological changes begin to reverse or at least do not progress. However, since the autoimmune mechanism is not prevented, changes in the stomach and the risk of developing stomach cancer remain.

**Aplastic Anemia**

Aplastic anemia includes a group of congenital and acquired diseases characterized by disruption of the hematopoiesis in the bone marrow, inhibition of the proliferation and differentiation of blood cells, and the development of pancytopenia in the peripheral blood. Two main etiological factors are involved in its development:

• genetic abnormality of stem cells (hereditary hypo-aplastic anemias)

• immunosuppression of bone marrow precursor cells (acquired hypo- and

aplastic anemia)

Hereditary aplastic anemia can be total or partial. Total aplastic anemia is characterized by *pancytopenia* (reduction of all blood cells) and *panmyelopathy* (reduction of all cellular elements of the bone marrow), while partial aplastic anemia is characterized by a deficiency of only erythroid cells in the bone marrow. Hereditary forms include Fanconi anemia, Estren-Dameschek anemia (total-aplastic), Blackfen-Diamond anemia (partial-aplastic), Ehrlich syndrome (hypoplastic anemia).

Acquired hypo- and aplastic anemias are caused by a number of chemical and pharmacological substances (antibiotics, sulfanilamides, cytostatic drugs, etc.), ionizing rays, as well as some infectious factors (herpes virus, tuberculosis, etc.), and develops against the background of autoimmune (systemic lupus erythematosis, rheumatoid arthritis ) and endocrine diseases (dysfunction of the thyroid gland, thymus).

***True erythrocytic aplasia*** is characterized by damage to only erythroid precursor cells in the bone marrow. In severe cases, these cells completely disappear from the bone marrow. The disease can develop along with neoplasias, especially thymoma and leukemia (tumor cells have a suppressive effect on bone marrow cells), after taking certain drugs, against the background of autoimmune diseases and under the influence of parvovirus infection. Except for parvovirus infection, the finding of autoantibodies against erythropoietin in the blood in most cases indicates that this pathology develops with an autoimmune mechanism.

***Parvovirus B19*** takes a special place in the development of erythroid aplasia. It is a DNA containing pathogenic virus from the parvovirus family. After penetration into the body Parvovirus B19 multiplies in erythroid cells and causes their destruction. If the infected person has no other diseases, aplasia of the bone marrow is a transient, recovery occurs within 1-2 weeks. However, short-term cessation of erythropoiesis in people with moderate or severe hemolytic anemia leads to aggravation of anemia and aplastic crisis.

The clinical picture of aplastic anemia is characterized by *anemic* and *hemorrhagic syndromes*. The onset of the disease is asymptomatic. Initial manifestations are associated with the destruction of erythroid line of cells, but eventually pancytopenia develops. Anemia causes weakness, paleness and shortness of breathing. Thrombocytopenia results in petechiae and ecchymoses. Neutropenia often and persistently causes infectious diseases, fever and general weakness. ESR increases to 40-60 mm/h. Splenomegaly is not observed.

**The following changes are observed in the blood:**

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| * pancytopenia: erythro-, leuko- and thrombocytopenia * decreasing of hemoglobin concentration to 20 - 30 g/l * the number of erythrocytes is 0.7 - 2.5 x 1012/l * persistent reticulocytopenia (reticulocytes ˂1%) * normochromia, macrocytosis, anisocytosis, poikilocytosis * increasing of the serum iron level |

The bone marrow biopsy and histological examination of samples is a great importance for diagnosis of aplastic anemia. The number of hematopoietic cells in the punctate of the bone marrow is very small and mostly it consists of lymphocytes, the significant advantage of adipose tissue in the bone marrow is revealed.

It is important to differentiate aplastic anemia from diseases such as leukemic leukemia and MDS, which manifest with the same clinical signs (especially pancytopenia). In aplastic anemia, the bone marrow is usually significantly deficient in cells, whereas in myeloid neoplasia, the bone marrow is rich in neoplastic cells.

Bone marrow transplantation is the preferred treatment for aplastic anemia. When a suitable donor cannot be found, there is usually a positive response to immunosuppressive therapy.

**Hemolytic Anemia**

Hemolytic anemia is a group of anemias characterized by premature, rapid breakdown of erythrocytes - hemolysis, caused by hereditary and acquired factors. Hemolysis can occur inside the blood vessels or outside (in the spleen, liver, bone marrow macrophages). Hereditary hemolytic anemias are caused by genetic mutations that associated with the abnormality in the structure and functions of erythrocytes. Acquired hemolytic anemias are caused by various exogenous (hemolytic poisons, high temperature, radiation) and endogenous (antibodies, activation of the complement system, etc.) factors on normal erythrocytes.

Although hemolytic anemias develop for various reasons, there are common symptoms that belong to all of them. These include premature destruction of erythrocytes and a life expectancy of less than 120 days, an increase in the level of erythropoietin in the blood and a compensatory acceleration of erythropoiesis, accumulation of hemoglobin breakdown products in tissues due to hemolysis.

*Extravascular hemolysis* causes activation of macrophages in spleen, liver and bone marrow, their hyperplasia and splenomegaly. The level of *free (unconjugated) bilirubin in the blood increases, jaundice develops.* The colloid state of bile is disturbed that leads to the development of cholelithiasis.

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| Clinical sings of extravascular hemolysis |
| 1) anemia 2) splenomegaly 3) jaundice |

The causes of *intravascular hemolysis* are mechanical damage to erythrocytes (pathology of heart valves, long term running, microthrombosis), activation of the complement system, intracellular parasites (malaria), exogenous toxic factors (toxins, snake venoms, insects), etc.

One of the important signs in intravascular hemolysis is a decrease in the level of *haptoglobin* (synthesized by the liver) in the blood. Free hemoglobin released from hemolyzed erythrocytes rapidly combines with haptoglobin and forms a complex that is captured by phagocytes, the level of haptoglobin decreases sharply. After haptoglobin is depleted in the serum, free hemoglobin is converted to brown methemoglobin. Hemoglobin and methemoglobin are excreted in the urine, giving the urine a red-brown color. Free hemoglobin and after a few days, hemosiderin are determined in the urine. Thus, *hemoglobinuria and hemosiderinuria* typical for intravascular hemolysis.

The iron released from hemoglobin accumulates in the renal tubules and causes the development of renal hemosiderosis. Heme groups separated from hemoglobin-haptoglobin complexes are broken down by phagocytes and turn into free bilirubin and jaundice occurs. Unlike extravascular hemolysis, *splenomegaly is not observed.*

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| Clinical sings of intravascular hemolysis |
| 1) anemia 2) hemoglobinemia 3) hemoglobinuria  4) hemosiderinuria 5) jaundice |

Hypoxia that develops during hemolytic anemias, stimulates the proliferation of erythroid cells in the bone marrow by increasing the production of erythropoietin. Enhancement of erythropoiesis causes *reticulocytosis* in peripheral blood. Therefore, hemolytic anemias belong to the group of hyperregenerative anemias. As anemia persists, extramedullary hematopoiesis occurs in the liver, spleen, and lymph nodes.

**Thalassemia**

Thalassemia, the most common form of hereditary hemolytic anemia, is endemic to the Mediterranean regions, the Middle East and the Indian subcontinent, Africa, and Asia. Azerbaijan is also included in this endemic zone. The prevalence of thalassemia and sickle cell anemia is protective against malaria and some intracellular microbes.

Thalassemia includes a group of diseases caused by inherited mutations that limit HbA synthesis. The erythrocytes of healthy people have the following types of hemoglobin.

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| HbA or HbA1 (ααββ) - 96%  HbA2 (ααδδ) - ≤ 3%  HbF (ααγγ) - ≤ 1%(fetal hemoglobin) |

Each α-chain of Hb is encoded by two α-globin genes on chromosome 16, and each β-chain is encoded by one β-globin gene on chromosome 11. As a result of the mutation, α- or β-thalassemia develops, depending on the disruption of the synthesis of α- or β-globin chains.

**↓α-globin→↓Hb→microcytic/hypochromic anemia → α-thalassemia**

**↓β-globin→↓Hb→microcytic hypochromic anemia→β-htalassemia**

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| In α-thalassemia:  HbBart-(γγγγ) - newborns  HbH (ββββ) - adults |
| In β-thalassemia:  HbA2 (ααδδ) - ≥ 3% |

A decrease in the synthesis of one type of globin chain results in hemoglobin deficiency (*hypochromia and microcytosis*) and also a relative abundance of another globin chain (intraerythrocyte inclusions).

***α-Thalassemia*** is characterized by reduced or complete cessation of synthesis of α-chains. There are normally 4 α-globin genes, and the type of α-thalassemia depends on how many α-globin genes are mutated. Anemia develops as a result of the lack of an adequate amount of hemoglobin, as well as the effect of an excess of unpaired β-, γ- and δ-chains. A mutation of 4 α-globin genes means a complete stop of α-globin chain synthesis. This leads to the synthesis of *HbBarts (γγγγ) tetramers* in newborns and ββββ-tetramer known as *HbH* in older children and adults. Clinical syndromes are classified according to how many α-globin genes are mutated (deleted).

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| α-thalassemia minimal (latent carrier) – 2% HbH - mutation of one α-globin gene |
| α-thalassemia minor (minor thalassemia)–5-6% HbH - mutation of two α-globin genes |
| α-thalassemia intermedia (H-hemoglobinopaty)– 40%HbH –mutation of three α-globin genes |
| α-talassemiya major (hidrops of fetalis) – 80-90%- HbBarts - mutation of four α-globin genes |

***Latent carrier (α-thalassemia minimal)*** – due to the deletion of 1 α-globin gene, a small decrease in the synthesis of α-chain is observed. Such individuals do not have symptoms of anemia, but mild microcytosis is observed.

***α-Thalassemia minor*** (α-thalassemia minor). It is due to deletion of two α-globin genes on the same chromosome (α/α -/-) or one α-globin gene on each of two chromosomes (α/- α/-). Both genotypes are clinically identical. The clinical picture of α-thalassemia minor is the same as β-thalassemia minor. The main symptom is microcytosis. Symptoms of anemic syndrome are minimal or undetectable.

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| Molecular analysis - determination of HbH is necessary to confirm the diagnosis. During hemoglobin electrophoresis, the HbA2 level is normal or low. |

***H-Hemoglobinopathy***  (α-thalassemia intermedia). This disease is caused by the deletion of three α-globin genes. Synthesis of α-chains is markedly reduced and β4-tetramers are formed. Because HbH has a high affinity for oxygen, it is unsuitable for delivering oxygen to tissues. This leads to tissue hypoxia. HbH is prone to oxidation, which causes its deposition inside the erythrocyte, the formation of intracellular aggregates, and accelerated hemolysis of erythrocytes in the spleen.

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| Decreasing of Hb, MCH and MCV and normal/decreased HbA2 typical for these patients. Molecular analysis found different levels of HbH (HbH up to -40%). |

Patients receive regular blood transfusions, which increases the risk of overloading the body with iron.

***Hydrops fetalis*** (α-thalassemia major). This is the most severe (lethal) form of α-thalassemia caused by the deletion of four α-globin genes. HbBarts (γ4) are formed in the fetus. *The main symptom of Barts syndrome is the presence of HbBarts in the fetal blood and the complete absence of HbF.* As a result of hemolysis, severe hypoxia develops in the fetus. From the third trimester of pregnancy, the main symptoms such as hepatosplenomegaly and diffuse edema are revealed. For compensation of hypoxia, extramedullary (in the liver) hemopoiesis is enhanced. As a result, liver dysfunction develops and albumin synthesis decreases sharply. This leads to a decrease in oncotic pressure and generalized edema.

If Barth's syndrome is detected during prenatal diagnosis, the pregnancy is terminated with the consent of the mother.

***β-Thalassemia*** is caused by mutations that limit the synthesis of β-chains. The severity of the disease varies according to the heterogeneity of the mutations. Mutations that cause β-thalassemia fall into two categories:

• β° mutations due to lack of synthesis of β-chains

• β+ mutations, characterized by a decrease in the synthesis of β-chains.

Disruption of the synthesis of β-chains leads to the development of anemia by two mechanisms: *ineffective erythropoiesis* and *extravascular hemolysis.* A decrease in hemoglobin (HbA) synthesis leads to the formation of *microcytic hypochromic* erythrocytes. An imbalance occurs in the synthesis of α- and β-chains. Unpaired α-chains are deposited inside erythrocyte precursor cells and form insoluble derivatives. This results in damage to the erythrocyte membrane. 70-85% of erythrocytes with damaged membranes undergo apoptosis right up to the bone marrow. This is called ineffective erythropoiesis. The erythrocytes undergo extravascular hemolysis in the spleen prematurely.

Another complication of ineffective erythropoiesis is that it stimulates excessive absorption of iron from the gut. Ineffective erythropoiesis reduces the level of hepcidin. Continuous blood transfusions and low hepcidin levels lead to excess iron in the body. As a result, secondary damage of parenchymal organs, hemochromatosis develops.

Clinical classification of β-thalassemias also depends on the level of genetic defect (β+ or β°) and homo- or heterozygosity. Pationts with mutations in two alleles of the β-globin gene (β+/β+, β+/β° or β°/β°) have severe transfusion-dependent anemia – *β-thalassemia major*. Heterozygotes with one mutated β-globin gene and one normal gene (β+/β or β°/β) usually have mild asymptomatic microcytic anemia. This is called *β-thalassemia minor* or β-thalassemia carrier.

***β-thalassemia major***. Anemia manifests itself 6-9 months after birth, because during this period excess hemoglobin synthesis switches from HbF to HbA.

If blood transfusion is not performed, the level of hemoglobin in patients is 3-6 g/dl. In erythrocytes, HbA is completely absent (genotype β°/β°) or is present in small amounts (genotypes β+/β+ or β+/β°). The level of hemoglobin HbF in erythrocytes increases markedly. At the same time, the amount of HbA2 increases.



In blood smear, *anisocytosis and poikilocytosis*, as well as *microcytosis* and *hypochromia* are observed. *Target-like cells, basophilic granularity*, and *fragmented erythrocytes* are also common. Due to ineffective erythropoiesis, the number of reticulocytes increases.

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| Blood smear shows target-like cells, microcytes, hypochromia, sometimes nucleated erythrocytes  Elevation of HbA2 and HbF is determined in hemoglobin electrophoresis.  HbA is low or absent |

*Hemosiderosis* and *secondary hemochromatosis* are noted in almost all patients. Excess iron damages the heart, liver and pancreas.

If children do not receive blood transfusions, they are stunted and die from anemia at an early age. The only treatment is a bone marrow transplant. DNA analysis of amniotic fluid cells is performed for prenatal diagnosis.

***β-Thalassemia minor*** is more common than thalassemia major and is common in the same ethnic groups. Most individuals are heterozygous carriers of the β+ or β° allele. Symptoms of the disease are often absent or mild. Peripheral blood smear reveals typical erythrocyte abnormalities - *microcytosis, hypochromia, basophilic granules and target-like cells.* Mild erythroid hyperplasia occurs in the bone marrow.

During electrophoresis of hemoglobin, a slight decrease in HbA, an increase in the level of HbA2 to 4-8% (normally 2.5 ± 0.3%) is detected. HbF level is usually normal, sometimes HbF is slightly elevated (2%, normal 1%).

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| β-thalassemia minor (β/β+), the mildest form of β-thalassemia minor, usually has an asymptomatic course with an increase in the RBC count.  • Microcytosis, hypochromia target-like cells in blood smear  • Hemoglobin electrophoresis, a slight decrease in HbA,  HbA2 (5%, normal 2.5%) and HbF (2%, normal 1%) are characteristic |

Confirming the diagnosis of β-thalassemia minor is important for two reasons: to differentiate thalassemia from microcytic-hypochromic iron deficiency anemia and to refer the patient for genetic counseling. Iron deficiency is usually ruled out after assessment of iron and serum ferritin levels and total iron binding capacity. Unlike iron-deficiency anemia in thalassemias, serum iron and ferritin are high, and serum total iron binding capacity is low.

Azerbaijan is one of the endemic zones where thalassemia is widespread. Therefore, in order to prevent thalassemia in Azerbaijan, since 2015, the "State Program on Combating Thalassemia" has been adopted. Within the framework of the relevant program, compulsory examination of persons entering into official marriage, detection of couples with both carriers of thalassemia, genetic counseling and prenatal diagnosis are applied to all pregnancies in such couples, and the genotype of the baby to be born is determined in advance. Although the goal of the program is to terminate pregnancies in which a sick fetus is detected, parents are free to make this decision.

Prenatal diagnosis is carried out in the 16-18 weeks of pregnancy. 10-20 ml of amniotic fluid is collected through transabdominal amniocentesis. Amniotic fluid is centrifuged to obtain a cell suspension consisting of amniocytes, and DNA molecular diagnostics is performed to determine the presence of thalassemia and its genotypic type.

**Sickle Cell Anemia**

Sickle cell anemia is a fairly common autosomal recessive (codominant) inherited hemoglobinopathy. Sickle cell anemia is caused by a mutation in the β-globin gene of hemoglobin, in which the amino acid glutamine (hydrophilic) is replaced by valine (hydrophobic) in the β-globin chain. At this time, hemoglobin with changed physical and chemical properties (HbS-α2βs2) is formed. In homozygous patients, more than 90% of hemoglobin consists of HbS.

HbS molecules polymerize when deoxygenated. During continuous deoxygenation, HbS aggregates precipitate in erythrocytes to form long, needle-like fibers, resulting in sickle-like erythrocytes.

The main pathological manifestations of sickle cell anemia are: *chronic extravascular hemolysis, occlusion of microvessels, tissue ischemia.* Since sickled erythrocytes are not resistant to mechanical stress, some degree of intravascular hemolysis also occurs.

Bone marrow becomes hyperplastic as a result of compensatory erythroid hyperplasia. Bone marrow expansion leads to bone resorption and skull deformities. Hemolysis leads to hyperbilirubinemia and the formation of pigmented gallstones. Chronic vascular occlusion of erythrocytes results in *splenic infarction - autosplenectomy*. During the course of the disease, various vasoocclusive crises (*pain crises)* are observed. *Acute chest syndrome* is a dangerous type of vasoocclusive crisis with lung damage.



In sickle cell anemia, *sickle-like cells, reticulocytosis and target-like cells* are found in the peripheral blood, moderately severe anemia develops (hematocrit 18-30%). These cells are not visible in the blood smear of carriers.

Diagnosis is based on clinical signs and the presence of *irreversible sickle-shaped red* blood cells. The diagnosis is confirmed by various tests that detect HbS. Blood samples are mixed with oxygen scavenging reagents (eg, metabisulfite), sickle cells are formed if HbS is present in the blood. *HbS-diagnosis is confirmed by the electrophoresis method.* Prenatal diagnosis is possible by analysis of fetal DNA obtained by amniocentesis or chorionic biopsy.

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| Results of laboratory examination:  • sickle cells and target cells are detected in the blood smear.  • The metabisulfite test causes sickling of cells with any amount of HbS in the blood, the test gives a positive result in both patients and carriers  • Hb electrophoresis confirms HbS and its quantity.  • In patients - 90% HbS, 8% HbF, 2% HbA2 (no HbA)  • In carriers - 55% HbA, 43% HbS, 2% HbA2 |

**Hereditary Spherocytosis**

Hereditary spherocytosis (Minkowski-Shoffar disease) is caused by genetic defects of the membrane skeleton of erythrocytes. This includes proteins that ensure the stability of the membrane skeleton - ankyrin, spectrin, band3, band4.2, etc. due to mutation of coding genes. Erythrocytes lose their membrane components and become in a spheroid shape. When spherocytes pass through the splenic sinuses, they are delayed and hemolyzed by macrophages, their lifespan is reduced to 10-20 days on average.

The disease can manifest itself in different forms depending on the degree of severity. Sometimes it manifests itself directly in the newborn period with acute hematological symptoms, but in 20-30% of cases the disease is practically asymptomatic. In moderately severe forms, the stable course of the disease is aggravated by aplastic and hemolytic crises. Parvovirus19 infection increases the risk of these crises.

Diagnosis is based on anamnesis, hematological and laboratory results. As a result of loss of potassium and water in spherocyte-shaped erythrocytes, the average concentration of hemoglobin (MHC) increases, and the osmotic resistance of spherocytes decreases sharply. This is determined by tests showing rapid osmotic lysis of spherocytes in hypotonic solutions.

In peripheral blood smear, *spherocytosis, abnormally small central pale area, hyperchromic erythrocytes* are determined. Spherocytes are a non-pathognomonic sign of hereditary spherocytosis, as spherocytes may also be present in autoimmune hemolytic anemia. Other features of hereditary spherocytosis are signs common to all hemolytic anemias – *reticulocytosis, erythroid hyperplasia of the bone marrow, splenomegaly, hemosiderosis, jaundice, cholelithiasis* (in 40-50% of cases).

Splenectomy is performed for treatment. Splenectomy prevents extravascular hemolysis, but spherocytosis persists. In addition, splenectomy increases the risk of sepsis in the patient.

**Hereditary glucose-6-phosphate dehydrogenase deficiency (G6PD)**

Erythrocytes are sensitive to the effects of exogenous and endogenous oxidants. Hereditary deficiency (X-recessive) of the enzyme glucose-6-phosphate dehydrogenase reduces the ability of erythrocytes to defend against oxidants and causes hemolysis.



Episodes of hemolysis, characteristic of G6PD deficiency, are caused by factors produced during oxidative stress. Various infections (viral hepatitis, pneumonia, typhus), drugs (antimalarial primaquine, chloroquine, sulfonamides) and certain foods induce this process. Horse fava (Vicia faba) is better known as a food item that stimulates hemolysis in these patients. During its digestion, oxidizing substances are formed in the blood and a hemolytic crisis occurs (favism).

G6PD deficiency causes both *intravascular* and *extravascular hemolysis*. During G6PD deficiency, increased H2O2 levels in erythrocytes cause hemoglobin denaturation and the formation of membrane-bound protrusions known as *Heinz bodies*. Erythrocyte membranes are damaged and intravascular hemolysis occurs. As these erythrocytes pass through the splenic sinuses, macrophages “bite” the Heinz bodies, producing abnormally shaped erythrocytes. Less damaged cells take on a spherical shape. Both "bitten" cells and spherocytes are captured by splenic macrophages.

Acute intravascular hemolysis, manifested by *anemia, hemoglobinemia,* and *hemoglobinuria,* usually occurs 2-3 days after exposure to oxidants. Hemolysis occurs mainly in senescent erythrocytes, and the hemolysis reaction is self-limiting with their depletion. Reticulocytosis develops during the recovery phase. Since hemolysis occurs episodically in G6PD deficiency, symptoms of chronic hemolytic anemia such as splenomegaly and cholelithiasis are not observed.

**Immune hemolytic anemias**

Immunohemolytic anemias are characterized by binding of antibodies (IgG or IgM) and/or complement to the membrane of erythrocytes and premature hemolysis of these erythrocytes. In this type of hemolytic anemia, the formation of antibodies is primarily - *idiopathic, or secondary to autoimmune processes* (SLE - systemic lupus erythematosus), lymphoproliferative diseases (CLL - chronic lymphocytic leukemia, lymphoma), sometimes as a result of various exogenous substances - drugs (penicillin, α-methyldopa ), can be caused by toxins, viral infections, etc. Antibodies involved in the development of immune hemolysis are also, divided into two groups: *warm antibodies* with maximum activity at normal body temperature – IgG, and *cold antibodies* with maximum activity in cold environment (relatively cold areas of the body) IgM.

Depending on the etiological factor, these anemias are classified as isoimmune, autoimmune, immune (or heteroimmune) hemolytic anemias.

*Extravascular hemolysis* usually occurs during *IgG-mediated reactions*. IgG is maximally active in the central regions of the body, at relatively warm temperatures, and binds to the membrane of erythrocytes (warm agglutinins). Erythrocytes coated with surface IgGs are recognized by splenic macrophages and undergo hemolysis. This type of immune hemolysis is caused during SLE, CLL and use of various drugs (classically, penicillin and cephalosporins). Drug may attach to RBC membrane (e.g., penicillin) with subsequent binding of antibody to drug-membrane complex. Drug may induce production of autoantibodies (e.g., α-methyldopa) that bind self antigens on RBCs.

*IgM-related reactions*, often cause *intravascular hemolysis*. IgM (cold agglutinin) is more active in relatively cold parts of the body (extremities) and binds to the membrane of erythrocytes and fixes the complement, but cannot completly activate it (complement is activated at >370C). When these erythrocytes circulate with the blood to warmer areas of the body, IgM is released from the erythrocyte surface, but the C3b component remains on their surface. This leads to complement activation and intravascular hemolysis.

*Direct and indirect Coombs tests* are used to diagnose immunohemolytic anemia:

• in the *direct Coombs test*, the patient's erythrocytes are mixed with a ready sample of serum. This sample serum contains specific antibodies against human Ig or components of the complement system. If the surface of the patient's erythrocytes contains Ig or a component of the complement system, antibodies cause their agglutination. Visually - visible cell aggregates are formed.

• In the *indirect Coombs test*, the patient's serum is mixed with a specially prepared sample. In this sample, there are erythrocytes coated with certain antigens. If there are antibodies against these antigens in the patient's serum, the agglutination reaction occurs.

***Hemolytic disease of the newborn*** (*isoimmune hemolytic anemia*) is a disease that causes hemolysis of the erythrocytes of the fetus and newborn due to the incompatibility of the erythrocyte antigens of the blood of the mother and the fetus. This disease develops as a result of Rh factor incompatibility (Rh-conflict) or incompatibility of group antigens (ABO) of the blood between the mother and fetus.

*Rh-conflict* occurs when a Rh-negative Rh(-) pregnant woman carries a Rh-positive (Rh+) fetus. *ABO-conflict* develops when the woman’s blood is “O” group, and the fetus blood is “A” (in 2/3 cases) or “B” (in 1/3 cases) groups. Antenatal and postnatal diagnosis of the disease is available.

*In antenatal diagnosis*, erythrocyte antigen incompatibility of parents, obstetrical-gynecological examination of the mother and anamnesis (previous abortions, birth of death child, miscarriages, cases of blood transfusion without taking into account the Rh factor) are investigated. During pregnancy, the titer of *anti-Rhesus antibodies* is determined in the blood of a Rh(-) woman. Detection of antibodies in the blood of a pregnant woman indicates the possibility of the disease in the fetus. If the risk of immune conflict is determined, amniotic fluid obtained by transabdominal amniocentesis and is examined for the concentration of bilirubin, proteins, glucose, iron, copper, and Ig. In the ultrasound examination, thickening of the placenta, edema, its rapid growth, polyhydria and hepatosplenomegaly indicate hemolytic disease.

*Postnatal diagnosis* is based on clinical manifestations (jaundice, anemia, hepatosplenomegaly) that appear immediately after birth or some short period after birth. At this time, laboratory findings as, increased unconjugated bilirubin, erythroblastosis, reticulocytosis, positive Coombs test - confirm the diagnosis.

**Hemolytic anemia caused by mechanical damage to**

**erythrocytes**

Mechanical hemolysis of erythrocytes can be caused by artificial heart valves, marathon (long term) running, and during thrombotic microangiopathy. In the blood of patients with artificial heart valves a result of the pressure gradient, hemolysis occurs under the influence of turbulent blood flow and repulsive forces. Microangiopathic hemolytic anemia develops in disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), SLE, and malignancy. In these diseases, as a result of deposition of fibrin and platelets on the wall of microvessels, their lumen is narrowed. Changes in the vascular wall cause hemolysis of erythrocytes passing through it.



Peripheral blood smear during mechanical hemolysis is characterized by severe *poikilocytosis - schizocytes, spiky, helmet-like, triangular erythrocytes*.

**Paroxysmal nocturnal hemoglobinuria**

Paroxysmal nocturnal hemoglobinuria is a disease caused by an acquired mutation of the PIGA gene, which encodes the synthesis of phosphatidylinositol (PIG), that is an intramembrane glycolipid. This is the only type of hemolytic anemia caused by an acquired genetic defect. The mutant gene (PIGA) is located on the X chromosome, and the mutation disrupts PIG synthesis. This protein ensures the fixation of proteins that normally inactivate complement on the cell’s membrane. As a result of its deficiency, complement-inactivating proteins are not fixed on the cell surface, and a clone of erythrocytes those sensitive to complement activation is formed. As a result, complement-dependent *intravascular hemolysis* occurs.

Hemolysis in patients is paroxysmal and nocturnal in only 25% of cases. Chronic hemolysis (without severe hemoglobinuria) is more typical for these patients. Nocturnal hemolysis is associated with a slight decrease in blood pH at night time, during sleep. Blood pH decreases at night due to the increasing of CO2, and it increases the activity of the complement system. The degree of anemia varies from mild to moderate. Thrombosis is the main cause of death in patients with paroxysmal nocturnal hemoglobinuria. Patients suffer from thrombosis of liver and cerebral vessels.

The laboratory diagnosis of paroxysmal nocturnal hemoglobinuria is confirmed by the *flow cytometry method*, which allows identifying erythrocytes with a deficiency of PIG-related proteins.