METHODS FOR DIAGNOSING BLOOD IN CLINICAL PRACTICE

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Hematological diagnostic methods are traditionally the most widely used methods for analyzing and testing blood in clinical diagnostic laboratories. The most important diseases diagnosed by hematological tests are anemias and hematopoietic disorders. Hematological tests are used to evaluate the response of the body to many diseases, assess disease severity, and determine the effectiveness of treatment.

The most commonly used methods for diagnosing blood in hematology include: morphological examination of erythrocytes in a blood smear, counting reticulocytes, measuring the osmotic resistance of erythrocytes, examining platelets in a blood smear, morphological examination of leukocytes, cytokine reactions, and bone marrow biopsy.

The most commonly used clinical blood tests for measuring the quantity and quality of blood cells include: hemoglobin concentration, color index, number of erythrocytes, number of leukocytes, leukocyte differential count, description of the morphology of blood cells, erythrocyte sedimentation rate, and number of reticulocytes and platelets.

Erythrocyte morphology is examined by increasing magnification with immersion up to x1000. The volume, color, shape, intensity of color, and presence of inclusions are evaluated. A normal erythrocyte is called a normocyte, has a diameter of 7.2-7.5 μm, and is a biconcave disk; a normochromic erythrocyte is characterized by intense staining of the peripheral cytoplasm and central pallor.

Modern automated hematology analyzers provide reliable clinical information on the state of the hematopoietic system and its response to various internal and external factors. High-tech hematology analyzers can measure more than 20 parameters and three histograms.

Hematology analyzers use impedance methods to measure erythrocytes and platelets, colorimetric methods to measure hemoglobin, and laser flow cytometry to measure leukocytes. Results are calculated based on the remaining parameters.

For a complete blood count, a blood sample is collected from the patient's vein using an anticoagulant-containing K-EDTA tube. Blood samples can be tested from 5 minutes to 1 hour after collection. The reliability of the results decreases if the analysis is performed more than 6-8 hours after sample collection.

The analyzer takes a sample of 15 μL or 11.7 μL of blood for a complete blood count. The sample is quickly and accurately diluted with an erythrocyte chamber. Diluting the sample is necessary to ensure a stable environment for counting and

measuring blood cells. Then, the sample is divided into two parts: one is further diluted and used with various reagents.

Leukocytes are differentiated in the channel, after lysis of erythrocytes and stabilization of leukocytes, a cytokine reaction occurs, and then leukocytes are distinguished by two markers: cell size and laser light scattering.

Basophils are differentiated from other granulocytes in the baso-channel. After being reprocessed with a unique lysate, all leukocyte cytoplasm goes through lysis except for basophils. Then, the channel measures the angle of light deflection at two degrees, which allows differentiation based on nuclear shape.

In summary, using tools that fully differentiate blood elements provides increased accuracy in diagnosis, allows for monitoring of trends and pathologies, and is useful in assessing the effectiveness of treatment.

Morphological characteristics of pathological erythrocytes.

1. Anisocytosis - the appearance of erythrocytes in various sizes. Normally, normocytes (with a diameter of 7-8 μm) make up 68-70% of peripheral blood, microcytes (with a diameter less than 6 μm) make up 15.5%, and macrocytes (with a diameter greater than 8 μm) make up 16.5%. When there are many microcytes, it is called microcytosis, and when there are many macrocytes, it is called macrocytosis. Erythrocytes with a diameter greater than 12 μm are called megalocytes.

2. Poikilocytosis - the presence of erythrocytes in various shapes. Poikilocytes can have different shapes, for example:

Ovalocytes are shaped due to defects in the membrane and are specific to hereditary ovalocytosis (hemolytic anemia), thalassemia, heavy iron deficiency anemia, and megaloblastic anemia.

Stomatocytes are erythrocytes with a mouth-shaped depression in the center of the cell. Stomatocytosis occurs after blood transfusion, liver disease, mononucleosis infection, or hereditary stomatocytosis (hemolytic anemia).

Spherocytes are erythrocytes that have lost their biconcave shape and have no central zone. If the diameter of spherocytes is less than 6 μm, they are called microspherocytes. Spherocytes occur in hereditary microspherocytosis (hemolytic anemia), jaundice, hemolytic transfusion reactions, artificial heart valve placement, and disseminated intravascular coagulation syndrome (DIC).

Acanthocytes are star-shaped erythrocytes. Acanthocytosis occurs in hereditary acanthocytosis (hemolytic anemia), lipoproteinemia, liver disease (cirrhosis), heparin treatment, and after splenectomy.

Elliptocytes are erythrocytes with a similar shape to an ellipse, and they have hemoglobin accumulation in the center of the cell. Elliptocytes occur in thalassemia (hereditary hemolytic anemia), heavy iron deficiency anemia, liver disease, and after splenectomy.

Anulocytes are erythrocytes with an empty center and a rim-like appearance. Anulocytes occur in iron deficiency anemia.

Drepanocytes are sickle-shaped erythrocytes and occur in sickle cell anemia.

Schistocytes are fragmented erythrocytes with small pieces. Schistocytes occur after jaundice, kidney transplantation, hemolytic uremic syndrome, DIC, and vasculitis.

Stomatocytes are erythrocytes with a mouth-shaped depression in the center of the cell. Stomatocytosis occurs after blood transfusion, liver disease, mononucleosis infection, or hereditary stomatocytosis (hemolytic anemia).

3. Anisochromia - the appearance of erythrocytes with different intensities of color. The color of red blood cells depends on the concentration of hemoglobin and normally constitutes 32-36% of the cell volume. Erythrocytes that are fully saturated with hemoglobin have a red color. Changes in erythrocyte color:

Hypochromia - erythrocytes with a pale color. Hypochromia occurs due to a decrease in the amount of hemoglobin in erythrocytes and is specific to iron deficiency anemia, glucose-6-phosphate dehydrogenase deficiency, sideroblastic anemia, and thalassemia. Hypochromia usually occurs with microcytosis.

Hyperchromia - erythrocytes that are overfilled with hemoglobin and appear darker. Hyperchromia is specific to vitamin B12 deficiency anemia, folate deficiency anemia, and hereditary spherocytosis (hemolytic anemia).

Polychromasia - the appearance of erythrocytes in various colors: bluish-purple, grayish-blue. These erythrocytes occur in vitamin B12 deficiency anemia, folate deficiency anemia, hemolytic anemia, and post-hemorrhagic anemia.

4. Erythrocyte inclusions. Normally, erythrocytes do not have inclusions in their cytoplasm.

Heinz-Ehrlich bodies are small inclusions (1-2 μm) located on the periphery of erythrocytes and consist of denatured hemoglobin. Heinz-Ehrlich bodies are found in some enzymopathies.

Basophilic punctuations in erythrocytes are residual mitochondria and RNA deposits located in diffuse blue spots. Basophilic punctuations can occur due to toxic damage to the bone marrow, such as exposure to heavy metal salts, radiation therapy, cytotoxic drug therapy, erythropoietic activation, megaloblastic anemia, and thalassemia.

Jolly-Gowell bodies are DNA remnants that are 1-2 μm in size and appear as reddish-purple, dumbbell-shaped inclusions in the cytoplasm of erythrocytes. Jolly-Gowell bodies can occur in megaloblastic anemia, hemolytic disorders, and after splenectomy.

Cabot rings are reddish-purple, ring-shaped inclusions located in the cytoplasm of erythrocytes. They are associated with exposure to heavy metal salts, megaloblastic anemias, and leukemias.

Schuffner granules are small reddish-purple dots located in the cytoplasm of erythrocytes and can be seen in peripheral blood three days after a malaria infection. Damaged erythrocytes increase in volume and lose their color.

Maurer's dots are large, round, purplish-red inclusions (10-15 dots of various sizes) found in erythrocytes of patients with tropical splenomegaly syndrome. Erythrocytes do not increase in volume or change color.

Siderotic granules are small (0.5-1.5 microns), blue-gray granules that contain iron (ferritin, hemosiderin) and are found in the cytoplasm of erythrocytes. They are detected by cytochemical tests and can be found in peripheral blood at a rate of 0.8-1.0%. Siderotic granules increase in number in sideroblastic anemia, myelodysplastic syndrome, hemolysis, and after splenectomy.

Reticulocytes are immature erythrocytes that indicate regenerative activity and the degree of erythropoietic activity. Reticulocytes can be used to diagnose hemolytic anemia, monitor therapy for iron deficiency anemia, vitamin B12 deficiency anemia, and folate deficiency anemia, monitor therapy with erythropoietin, and evaluate regeneration after cytotoxic therapy or bone marrow transplantation. Reticulocytopenia can be seen in paroxysmal nocturnal hemoglobinuria, leukemia, myelodysplastic syndrome, metastases to the bone marrow, aplastic anemia, vitamin B12 deficiency anemia, and pure red cell aplasia.

There are five classes of reticulocytes based on their morphology. The five forms of reticulocytes are as follows:

0 group: erythrocytes with a nucleus that is surrounded by a flattened reticulocyte.

1 group: erythrocytes with a centrally located reticulocyte.

2 group: erythrocytes with a reticulocyte that is dispersed throughout the cell.

3 group: erythrocytes with a reticulocyte that is located in one part of the cell.

4 group: erythrocytes with reticulocyte remnants located in the peripheral part of the cell.

In newborns, the number of reticulocytes is higher than in adults. The number of reticulocytes decreases to normal levels by four months of age. The percentage of reticulocytes in peripheral blood is 1%. The reticulocyte index is used to evaluate erythropoietic activity and is calculated as the number of reticulocytes per 1000 erythrocytes. In normal conditions, it ranges from 2-10‰ or 0.2-1%.

Reticulocytes indicate the regenerative capacity of erythropoiesis. In hemolytic anemias, reticulocyte counts increase sharply during a crisis. Reticulocytosis can also be seen in polycythemia, malaria, and during therapy for anemia. Certain drugs, such as iron preparations and vitamin B12 supplements, can increase the number of reticulocytes.

A decrease or absence of reticulocytes is a poor prognostic sign in anemia and indicates a loss of regenerative capacity (aplastic anemia).

The main component of erythrocytes is hemoglobin, which makes up 98% of their composition. Hemoglobin is composed of heme, which is a molecule containing an iron atom, and globin, which is a protein made up of four polypeptide chains. In humans, two of the polypeptide chains are alpha chains, while the other two are beta,

gamma, or delta chains. The ability of hemoglobin to bind to oxygen is dependent on the presence of four polypeptide chains. If all four chains are the same type, the hemoglobin molecule is prone to denaturation, which can shorten the lifespan of erythrocytes (hemoglobinopathy). Hemoglobin can exist in two forms in blood: oxygenated hemoglobin (hemoglobin with oxygen bound to it) and reduced hemoglobin (hemoglobin that has bound to carbon dioxide and bicarbonate). Oxygenated hemoglobin is found in arterial blood and gives it a bright red color, while reduced hemoglobin is found in venous blood and gives it a darker red color. One gram of hemoglobin can bind to 1.34 ml of oxygen – this is known as the Geftner coefficient.

There are three types of hemoglobin found in normal adults: Hb A (97%), Hb A2 (2%), and Hb F (1%). The amount of hemoglobin in newborns is typically between 140-190 g/L, but it can increase to 165-225 g/L after a few days. Hemoglobin levels decrease to normal levels by 4 months of age. In adults, normal hemoglobin levels range from 120-160 g/L for women and 130-180 g/L for men. Hemoglobin levels can be affected by certain factors such as living at high altitudes, intense physical activity, and after certain medical procedures. A decrease in hemoglobin levels is a common symptom of anemia and can be seen in conditions such as aplastic anemia and massive blood loss.

Hemoglobin can also exist in other forms, such as methemoglobin and carboxyhemoglobin. Methemoglobin is formed when the iron atom in heme is oxidized from Fe++ to Fe+++. Erythrocytes normally contain a small amount of methemoglobin (0.03-0.3 g/L), but this can increase in certain conditions such as congenital methemoglobinemia (due to a deficiency in the enzyme methemoglobin reductase) and congenital hemoglobin M methemoglobinemia (due to the production of abnormal hemoglobin M). Carboxyhemoglobin is formed when hemoglobin binds to carbon monoxide instead of oxygen. This can occur in situations where carbon monoxide is present, such as in cases of smoke inhalation or exposure to car exhaust fumes.

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