CLINICAL-LABORATORY DIAGNOSIS AND TREATMENT METHODS OF APLASTIC ANEMIA

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Aplastic anemia is a hematological syndrome characterized by peripheral blood pancytopenia and degeneration of red bone marrow cells and their microenvironment due to qualitative and quantitative changes. Aplastic anemia is a relatively rare disease, affecting 2-3 cases per 1 million individuals annually. It can occur in all age groups, but two peaks are observed - between 10-25 years old and 60 years old and above, mostly in females.

Approximately half of the patients with aplastic anemia have unknown causes, even after careful collection of medical history. The development of aplastic anemia can be attributed to various factors such as chemical agents (benzene and its derivatives, nitro compounds, lacquers, pesticides, etc.), ionizing radiation, drugs (antibiotics, sulfonamides, anticoagulants, etc.), bacterial and viral infections.

Currently, several mechanisms are known to be involved in the development and progression of aplastic anemia:

- Suppression of the proliferative activity of hematopoietic stem cells resulting in functional and anatomical damage.
- Destruction or dysfunction of the microenvironmental elements of the bone marrow.
- Dysregulation or suppression of hematopoiesis due to immunopathological conditions.
- Inadequate stimulation of hematopoiesis due to various factors.

A combination of different mechanisms can be involved in the pathogenesis of aplastic anemia.

Classification.

Currently, aplastic anemia is classified into the following forms:

- Mild: granulocytopenia >0.5×109 / L.
- Severe: granulocytopenia <0.5×109 / L, thrombocytopenia <20.0×109 / L.
- Very severe: granulocytopenia <0.2×109 / L.

To determine the severity of aplastic anemia, at least three peripheral blood tests are performed before starting treatment.

Refractory aplastic anemia is diagnosed when there is no response to combined immunosuppressive therapy after 6 months of treatment or after the second course of antithymocyte immunoglobulin.

The clinical presentation of aplastic anemia is associated with cytopenic and hemorrhagic syndromes. In most patients, the disease develops gradually with anemia syndrome and an increase in bleeding.

In 15% of patients, aplastic anemia starts suddenly and progresses rapidly, accompanied by severe nosebleeds, gingival bleeding, petechiae and ecchymosis, necrotic tonsillitis, and sometimes fever.

Visible signs of bleeding, such as bleeding in the oral mucosa or conjunctiva, may indicate significant bleeding in the body. In some patients, hemorrhagic manifestations are not detected during initial examination. Hemorrhagic symptoms, often accompanied by profound anemia, include general weakness, shortness of breath, palpitations, and pallor of the skin and mucous membranes.

Diagnostic criteria:

- Triad of pancytopenia - anemia (hemoglobin <110 g/l), granulocytopenia (granulocytes <2.0×109 / l), thrombocytopenia (platelets <100.0×109 / l);

- Decreased cellularity and absence of megakaryocytes in bone marrow aspirate (sternum puncture);

- Aplasia of bone marrow seen in bone marrow biopsy of the iliac crest (bilateral trephine biopsy).

The diagnostic stages of aplastic anemia are presented in the following scheme:

Complaints and anemia collection:
- anemia syndrome
- hemorrhagic syndrome
- having acute hepatitis six months before the disease;
- the presence of pain in the bones
- unprovoked fever in anemia
- Infectious diseases and used drugs over the past 6 months

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Physical tests:
- Structural anomalies on the face;
- the presence of pigmentation of the skin
- the presence of claws dystrophy
- enlargement of the lymph nods
- petechia and ecchymosis

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Laboratory studies:
UQT - anemia, thrombocytopenia,
leukopenia, EChT increase, neutropenia, lymphocytes, reticulotsitopenia in the formula for leukocytes

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Hematologist's vision

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Diagnosis confirmation

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Treatment tactics

Diagnostic evaluation for aplastic anemia:

List of mandatory medical examinations:

- Initial evaluation by a hematologist (examination, consultation);
- Cytological examination of bone marrow aspirate (calculation of bone marrow cellularity);
- Histological examination of bone marrow biopsy specimen;
- Complete blood count, including platelets and reticulocytes;
- Cytological preparation of bone marrow aspirate obtained by puncture;
- Obtaining a histological sample of bone marrow biopsy;
- Phenotyping, determination of blood group and Rh factor.

List of additional medical tests used when mandatory evaluation data is insufficient or treatment is ineffective:

- Determination of total bilirubin level in blood;
- Determination of direct and indirect bilirubin levels in blood;
- Determination of AST levels in blood;
- Determination of ALT levels in blood;
- Determination of g-glutamyltransferase levels in blood;
- Determination of alkaline phosphatase levels in blood;
- Monitoring of iron metabolism indices (serum iron, ferritin, total iron binding capacity, iron saturation of transferrin, transferrin);
- Determination of erythropoietin levels in blood;
- Direct antiglobulin test (Coomb's test);
- Indirect antiglobulin test (indirect Coomb's test);
- Coagulogram;
- Standard cytogenetic analysis;
- Fluorescence in situ hybridization (FISH);
- Immunophenotypic analysis of dysplastic changes using flow cytometry (Flow-Score);
- PCR for viral infections (viral hepatitis, cytomegalovirus, herpes simplex virus, Epstein-Barr virus);
- Cytogenetic analysis;
- HLA typing;
- Immunophenotyping of peripheral blood cells (erythrocytes, granulocytes, monocytes) for detection of clonal populations;
- Computed tomography of the thorax;
- Computed tomography of the head and neck;
- Ultrasonography of abdominal organs;
- Ultrasonography of peripheral lymph nodes;
- Ultrasonography of breast (in women);
- Ultrasonography of prostate gland (in men);
- Echocardiography.

Laboratory signs of aplastic anemia:

1. Peripheral blood:

- Pancytopenia (sharp decrease in the quantity of erythrocytes, thrombocytes, and leukocytes);

- Normochromic and normocytic erythrocytes;

- Relative lymphocytosis (decrease in the absolute quantity of lymphocytes, increase in the relative quantity in the leukocyte differential count).

2. Bone marrow examination shows a sharp decrease in all cell lines, with an observed increase in the quantity of lymphocytes.

Cytological differential diagnosis of anemias is provided in Table 2.

An example of complete blood count for aplastic anemia: hemoglobin - 42 g/l, erythrocytes - 1.3 × 1012/l, hematocrit - 20%, MCV - 110 fl, MCH - 31 pg, leukocytes 0.9 × 109/l. Leukocyte differential count: neutrophils 13%, lymphocytes 66%, monocytes 21%, platelets 20 × 109/l, ESR 65 mm/hour.

Differential diagnosis is performed based on the syndrome of hematopoietic suppression, including myelodysplastic syndromes, aplastic anemia, myelotoxic (associated with various severe endogenous and exogenous intoxications), consumption hematopoiesis (associated with disseminated intravascular coagulation syndrome), autoimmune cytolytic hematopoiesis (hematopoiesis suppression due to antibody formation), and other forms of hematopoietic suppression and temporary dysfunctional cytopenias, as well as intermediate forms of cytopenias (Table 1).

Table 1. Differential diagnosis of aplastic anemia

Diagnosis	Tests for differential diagnosis	Inspections	Criteria for diagnosis exclusives
Leukemia with acute lymphoblastic and myeloblast	Anemic and hemorrhagic syndrome, symptoms of intoxication	Red bone marrow puncation, i.e., myelogram and trepanobiopy	Characteristics of the red bone marrow punctiation and trepanobioptati: deep pancytopenia (anemia, leukopenia, thrombocytopenia). A sharp reduction in the three hemapoetic barrier rows, fat degeneration of the red bone marrow, without blast cells.
Megaloblast anemia	Erythrocytesandhemoglobinarereduced,creatingmildleukopenia.Mildhemolyticsyndromecanoccur	General blood analysis and myelogram	Aplastic anemia does not contain hyperchrome and macrocyte red blood cells, neutrophils hypersegmentation in the red bone marrow and peripheral blood; The myelogram shows a sharp decrease in the production of red blood cells, not hyperplasia of megaloblasts. There will be no damage to the nervous system
Myelodisplastic syndrome	Anemic and hemorrhagic	Myelogram	A sharp reduction in the hemapoetic barrier lines of the three is seen, and fat

	syndrome, symptoms of intoxication		degeneration of the red bone marrow is seen. Dizeritropoeticerythropoesis is not characteristic.
Paroxysmal night hemoglobinuria	Anemic, hemorrhagic and hemolytic syndrome, symptoms of intoxication	General blood analysis and myelogram, general weight analysis, PTG clone immunophenotype analysis in peripheral blood	In aplastic anemia, blood plasma does not contain high amounts of free hemoglobin and hemocydinuria, hemoglobinuria. Thrombosis is not characterized by localization of the kidneys and other organs and in the limbs. There will be no reticulotsitosis. The Hema and Gartman tests are negative. Three hemapoeticbarrier rows a sharp reduction, fat degeneration of the red bone marrow is seen.
Partsial red cell hypoplastic anemia	Anemic syndrome, intoxication symptoms	General blood analysis and myelogram, general weight analysis, PTG clone immunophenotype analysis in peripheral blood	Presence of hemorrhagic syndrome. A sharp reduction in the hemapoetic barrier lines of the three is seen, and fat degeneration of the red bone marrow is seen. PTG-clone analysis is negative.
Aggranulotsitosis	Anemic and hemorrhagic syndrome, symptoms of intoxication	Anemia. General blood test and examination of myelograms.	In aplastic anemia, acute septic condition and gectic temperature are rare. With the onset of the disease, erythrocytes, hemoglobin and platelets in peripheral blood decrease sharply. Full or partial leukopenia, except granulocytes. A sharp reduction in the hemapoetic barrier lines of the three is seen, and fat degeneration of the red bone marrow is seen.
Connective tissue systemic diseases (systemic reddish-brown, rheumatoid arthritis, etc.)	Symptoms of pancytopenia and intoxication	Anemia. General blood test and examination of myelograms.	A sharp reduction in the hemapoetic barrier lines of the three is seen, and fat degeneration of the red bone marrow is seen.
Chronic hepatitis and liver cirrhosis	Anemic and hemorrhagic syndrome, symptoms of intoxication	Anemia. General blood test and examination of myelograms. Viral hepatitis B, IFA for C,	Changes in indicators evaluating the functional state of the liver, hepatomegalia and splenomegalia are not characteristic. Characteristics of red bone marrow punction and

		PSR Analysis	trepanobioptati: deep pancytopenia (anemia, leukopenia, thrombocytopenia). A sharp reduction in the hemapoeticbarrier lines, fat degeneration of the red bone marrow.
Endocrine diseases: hypopituitarism and hypotheremism	Anemic syndrome, intoxication symptoms	Anemia. General blood analysis. Analysis of myelograms and trepanobiopsia, thyroid hormones.	There will be no hypothermia clinic. Characteristics of the red bone marrow punctiation and trepanobioptati: deep pancytopenia (anemia, leukopenia, thrombocytopenia). Sharp reduction of the three hemapoetic barrier rows, fat degeneration of the red bone marrow
Hypersplenism syndrome for various causes (infectious, parasitic, Goshe disease, Nimanna-Pica disease)	Pancytopic syndrome, symptoms of intoxication	Anemia. General blood analysis. Myelogram and trepanobiopsia	Splenomegalia is not characteristic. Sharp reduction of the three hemapoetic barrier rows, fat degeneration of the red bone marrow
Fanconi anemia	Anemic, hemorrhagic syndrome, symptoms of intoxication	Anemia. General blood analysis. Myelogram and cytogenetic analysis. Analysis of hormones. Checking high sensitivity of lymphocytes with diepoxybutan in peripheral blood	Congenital anomalies of skin pigmentation, internal organs and the bone system are not characteristic.
Anemia of poor-quality diseases	Anemic, hemorrhagic syndrome, symptoms of intoxication	Myelogram analysis	Lack of special cancer cells and sharp reduction of three-line hemapoetic barrier cells

In patients aged up to 20 years with severe aplastic anemia, hematopoietic stem cell transplantation from a suitable donor is considered the optimal treatment method to restore hematopoietic stem cells. This ensures long-term survival for more than 80% of patients. It should be emphasized that 20% of patients with aplastic anemia can have a suitable donor for hematopoietic stem cell transplantation as the first-line therapy. However, for most patients, immunosuppressive therapy is considered the main treatment due to the high risk of complications associated with transplantation, such as a history of multiple transfusions.

The "gold standard" for treating aplastic anemia, which yields the best results, is a combination of antithymocyte immunoglobulin and cyclosporine, taking into account the patient's age and severity of the disease. Cyclosporine is administered at a dose of

5 mg/kg per day for a long period (12-15 months or more) until remission is achieved. During therapy, it is important to monitor the drug's nephro- and hepatotoxic effects by regularly assessing biochemical indicators.

Antithymocyte immunoglobulin (Thymoglobulin) is administered intravenously at a dose of 2.5-3.5 mg/kg per day for a slow infusion over 8 hours. This treatment is performed in an aseptic environment for a duration of 5 days.

Monotherapy against combination immunosuppressive therapy is not only effective in severe aplastic anemia but also considered the most effective approach in average aplastic anemia, leading to sustainable remission in 60-80% of patients. However, a certain percentage of patients may be resistant to ongoing immunosuppressive therapy. In such cases, alternative immunosuppressive methods or hematopoietic stem cell transplantation should be considered. In recent years, new drugs with immunosuppressive effects and agents that stimulate hematopoiesis (such as monoclonal antibodies, TNF-alpha blockers, mycophenolic acid preparations, and others) have been used in the treatment of patients with aplastic anemia.(Table 2)

Table 2.Drug preparations used to treat aplastic anemia

Name of the international non-patented name	Average delivery frequency	Unit	Average daily dose	Average course dosage
Anti-tumor immunodepressant and supplemental preparation				
Antitimotsitar immunoglobulin	1,0	Vial	210	1050
Cyclosprorin	1,0	Tablet	350	126000

Splenectomy is performed in severe aplastic anemia only when other therapies have no effect. Patients are placed under dispensary surveillance. After starting the course of treatment with antithymocyte immunoglobulin in the hospital, treatment with cyclosporine is continued in an outpatient setting under the regular supervision of a hematologist, monitoring the following indicators:

- Complete peripheral blood analysis - once a week;
- Biochemical blood analysis (total protein, bilirubin fractions, urea, creatinine, aminotransferase, LDH, magnesium, sodium, potassium, calcium) - once a week;
- Coagulogram - once every 2 weeks;
- General urine analysis - once a week;
- X-ray examination, including CT of the chest;
- Bacteriological tests - with a constant increase in body temperature above 37.5-38.0 °C during the day;
- Determination of the cyclosporine level in blood cells - once a week in the first month of treatment, then once every 2-4 weeks;
- Detection of hepatitis B and C markers (ELISA and PCR) - once a month, then once every 3-6 months;
- Sternal puncture and trepanobiopsy - performed once every 6-12 months;

- Immunophenotyping of erythrocytes and granulocytes to detect PNH clone - once every 6-12 months.

The indications for hospitalization are primarily determined by the severity of the patient's condition and ongoing therapy. Hospitalization is necessary for treatment with antithymocyte immunoglobulin, splenectomy, and hematopoietic stem cell transplantation. Therefore, hospitalization is required for:

- Therapy with antithymocyte immunoglobulin;
- Splenectomy;
- Allogeneic transplantation of hematopoietic stem cells;
- Monitoring and adjustment of treatment.

Emergency hospitalization is required for:

- Newly diagnosed aplastic anemia;
- Febrile neutropenia;
- Hemorrhagic syndrome.

Timely initiation of therapy leads to a five-year survival rate of 80% in patients with aplastic anemia.

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