The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.

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INTRODUCTION

Hemolytic anemias are a group of diseases that differ in etiology, disease course and methods of treatment [1]. The term “hemolysis” understood to mean significant intra- or in-vascular decay of red blood cells (RBC), encompassing a wide range of laboratory and clinical conditions, whether congenital or acquired, as well as physiological or pathological. Significant and rapid decay of RBC hemolytic anemia develops, which manifested by a decrease in hemoglobin below the reference values and a compensatory increase in erythropoiesis [2]. Hemolytic anemias have different etiology and pathogenesis, but the clinical manifestations are quite similar. The main clinical manifestations of RBC hemolysis are hyperregenerative anemia of varying severity, jaundice against the background of increased bilirubin (due to indirect fraction) and hepatosplenomegaly [3, 4].

According to the classification, hemolytic anemia divided into hereditary and acquired. Hereditary anemias are divided into membranopathies that are associated with impaired protein synthesis in the RBC membrane (microspherocytosis, ovalocytosis, elliptocytosis, paroxysmal nocturnal hemoglobinuria); enzymopathy, as a result of impaired activity of RBC enzymes (G-6-PD deficiency, defects in the Embden-Meyerhof cycle, glycolysis, pentosephosphate cycle, nucleotide exchange, glutathione, methemoglobinemia). Hereditary hemolytic anemias also include hemoglobinopathies, which are associated with defects in the structure or synthesis of hemoglobin or heme protein - sickle cell anemia, unstable hemoglobin, thalassemia, porphyria [5,6]. Acquired hemolytic anemias include immune forms (autoimmune, transimmune, heteroimmune, haptic); anemia that develops due to the influence of infectious factors (viral - cytomegalovirus, Epstein-Barr virus, hepatitis C, B, E, HIV virus; bacterial - meningococcal, pneumococcal infection, on the background of sepsis; parasitic - toxoplasma). Anemia due to exposure to drugs, heavy metals and poisons; against other hematological diseases (leukemia, lymphomas, idiopathic thrombocytopenic purpura, megaloblastic anemia, after hematopoietic stem cell transplantation), other autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, rheumatoid arthritis, rheumatoid arthritis, rheumatoid arthritis)

Racial identity, burdened hereditary history and clarification of possible causes that led to the development of the disease have important diagnostic value in suspected hemolysis in children [8]. In childhood, hereditary hemolytic anemias are predominantly, accounting for more than 90% of cases. Whereas acquired (immune) hemolytic anemias occur in 1 - 3 cases per 100,000 child population per year [7,9]. A fundamental test for the diagnosis of immune hemolytic anemia is to determine the direct response to antiglobulin (DAT) and the direct Coombs test. It is possible to determine the immunoglobulin or complement on the surface of RBC with this test. Its value lies in the ability to differentiate the immune forms of hemolytic anemia from non-immune [10]. In autoimmune hemolytic anemia, antibodies are predominantly of the IgG class, with IgA or IgM being the activators of complement. RBC combined with immunoglobulins by phagocytosis removed from the bloodstream by spleen macrophages. Part of the RBC in combination with complement destroyed in the liver by phagocytosis induced by complement receptors in intracellular hemolysis. In 70% of cases, a direct response to antiglobulin (DAT) has a positive result. However, 30% of patients are DAT-negative, which complicates the diagnosis, observed in severe, with frequent recurrences of hemolysis, which is refractory to drug therapy, and in most cases completed by mortality [2, 11].

The main method of research in the diagnosis of hemolytic anemia is to study the morphology of RBC. However, morphological changes in RBC can occur in various forms of dis-
CLINICAL CASE
Child S. (boy) was born in 2016 year. Admitted to the newborn pathology department of Vinnitsa Regional Children's Clinical Hospital on the first day after delivery. It is known that the baby was born from 7 pregnancies, 5 delivery (1 miscarriage in the first trimester, 1 abortion), reported at gestation 37-38 weeks and weighing 3000 g. On objective examination revealed that the condition of the baby is severe, fever. The baby is sleepy, the mother's breasts are reluctant, appetite reduced. There was shortness of breath with the participation of ancillary muscles, respiratory rate 68 per minute. The skin is dry, pale with a yellowish tinge, without signs of hemorrhagic syndrome. Peripheral lymph nodes not enlarged. In the lungs, breathing is impaired, moist rales heard over the entire surface on both sides. Heart tones are rhythmic, with systolic murrmur over the top of the heart. Heart rate is 145 beats per minute. Abdomen is soft, painless. The liver enlarged, protrudes by 3 cm, the spleen by 3 cm from the edge of the costal arch. Diuresis in sufficient quantity, without pathological changes.

During the stay in the ward the following laboratory methods were performed: HB 102 g/l, RBC 3.1*10^{12}/l, reticulocytes 28 %, leukocytes 12.3*10^9/l, total bilirubin 266 µmol/l (direct 4 µmol/l, indirect 262 µmol/l), TOXO IgG-506, IgG CMV-13.7 opt.un, above the reference values. Ultrasound examination of abdominal organs revealed an increase in the size of the spleen to 52*26 mm. On the basis of complaints, anamnesis and laboratory methods of the study the child was diagnosed with intrauterine infection (IUI) of unspecified etiology with brain damage, lungs (bilateral pneumonia), liver, kidney. Intended treatment: prednisolone 9 mg/day, ampirsulbin - 100mg/kg / day, RBC № 1 - 10 ml/kg and maltofer - 3 mg/kg/day. At the age of 3 weeks after birth, the child in a satisfactory condition was discharged home.

By the age of 6 months, the child admitted to the onco-haematological department every 2 weeks with complaints of hemoglobin reduction to 50 g/l, pallor and yellowness of the skin, hepatomegaly and splenomegaly. During this observation period, the patient's CBC showed an increase in the level of reticulocytes, indicating a high degree of bone marrow regeneration (Table I). Hemolytic crisis, the child had osmotic resistance of RBC: min 0,24; max 0,24.

As a result of additional methods of examination the child at the age of 6 months was diagnosed with congenital hemolytic anemia, unspecified in the state of acute hemolytic crisis. During each hospital stay at the stage of treatment, the
child constantly required a replacement blood transfusion of RBC. In addition, in the treatment of hemolytic crisis, no positive response to corticosteroid therapy.

From 6 months to 3 years of age on a monthly basis, there was an acute hemolytic crisis, which manifested in the pale, yellowing of the skin and mucous membranes. At the time of hospitalization, the child was sluggish, adventurous, refused food, feverish. Objective examination showed pallor and yellowness of the skin and sclera, without signs of hemorrhagic syndrome. Auscultation of the lungs vesicular breathing, auscultation of the heart - a systolic murmur at the I point. Palpation was determined by the increase in the size of the liver, which protruded from the edge of the costal arch by 3 - 4 cm. Largely during the examination and treatment of the child was observed enlargement of the spleen - 5-6 cm from the edge of the costal arch (Fig. 1).

According to ultrasound data, the spleen size increased almost twice during this period. (Figure 2).

In order to clarify the etiology of hemolytic anemia and make a differential diagnosis, the patient underwent a series of laboratory examinations. In January 2017, studies of erythrocyte morphology were conducted, so was observed increase level of reticulocytes at the level of 350 %, MCV - 93.8 μm (N - 70.00-101.00), also expressed macrocytosis, polychromatophiles 5 - 6 in the field of view. In February 2018, moderate macrocytosis, single target cells and only a small number of Kebota rings detected morphologically. An examination of erythrocyte morphology in February 2019 revealed anizocytosis with a predominance of macrocytes, basophilic puncture of RBC, and the presence of Kebota rings and Jolly calves, which is characteristic of megaloblastic anemia.

Given the constant macrocytosis, the patient was determined to have levels of vitamin B 12 and folic acid, which were within the reference values: vitamin B12 - 233.3 pg/ml (N 191.0 - 663.0), folic acid - 6.8 ng/ml (N 4.6 - 18.7), which made it possible to eliminate megaloblastic anemia.

During the period of observation and treatment, the child as a result of hemolysis of RBC showed a constant increase in bilirubin due to the indirect fraction (table II).

In addition, bone marrow studies performed to make a differential diagnosis with other anemia. Against the background of normal cellularity of the preparations, there was marked hyperplasia of the erythrocyte sprout without disturbance of maturation (preserved cytoplasmic bridges). Myeloid sprout relatively narrowed without disturbance of ripening. Megakaryocytic sprout moderately narrowed. Dyspoise is not expressed. Hemophagocytosis not detected. Anaplastic cells not detected. Thus, the morphological data of the bone marrow study corresponded to hemolytic anemia.

![Fig. 1. Enlargement of the spleen - 5-6 cm from the edge of the costal arch](image)

![Fig. 2. Dynamic of the spleen size ground on ultrasound data](image)
To exclude fermentopathy, only glucose-6-FDG could be met, which was also within the reference values of 15.27 μmol/l, ferritin - 328 ng/ml (N - 28,0 - 397,0 ng/ml).

To exclude immune hemolytic anemia, a direct Coombs test was performed: January 2017 - negative, April 2018 - negative. To exclude acquired hemolytic anemia on the basis of infectious trigger factor, the child was examined for herpes infections: IgG CMV 8,763 opt/µl, IgG HSV-6 1,147 opt/µl, IgG EBV (EBNA) 0,227 opt/µl, IgM EBV 0,003 opt/µl, IgM CMV 1,232 opt/units, which made it possible to exclude hemolysis caused by herpetic infection. Studies on hepatitis B and C viruses and HIV antibodies were also negative.

Given the frequent replacement of blood transfusions by RBC, it is advisable to monitor the iron exchange rates that were within our patient’s normal range: serum iron - 32 μmol/l, ferritin - 328 ng/ml (N - 28,0 - 397,0 ng/ml).

To date, the cause of erythrocyte hemolysis remains unclear and the final diagnosis not established. The child diagnosed with hemolytic anemia of unknown origin.

CONCLUSIONS
1. Early development of hemolytic anemia in children characterized by a severe course with frequent hemolytic crises, difficult diagnosis and poor prognosis.
2. Patients with severe hemolytic crises occurring in the early age may need constant replacement transfusions of the blood.
3. Diagnosis of hemolytic anemia requires a wide variety of methods of investigation, which allow to establish the cause of hemolysis and to perform differential diagnosis.
4. The study of anamnestic data, morphological examination of peripheral blood smear and biochemical markers of hemolysis, conducting a Coombs direct test are obligatory diagnostic measures in patients with hemolysis at first contact.

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CONFLICTS OF INTEREST
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