16. Pedachenko E.H., Yarmoliuk E.S. Orfannыe y redkye zabolevanyia v neirokhyrurhyy: pryhlashenye k dyskussyy. Ukrayinskiy neyrohirurgichniy zhurnal. 2016; 2: 5-17. (In Ukrainiane).
17. Peterkova V.A., Semicheva T.V., Kuznetsova E.S. Vrozhdennaya disfunktsiya koryi nadpochechnikov u detey (etiologiya, patogenez, klinika, diagnostika, lechenie) : posobie dlya vrachey. 2003. 29-34. (In Russian).
18. Shkrobanets I.D., Lastivka I.V., Polishchuk M.I., Hodovanets Yu.D. Problemy orfannykh khvorob. Neonatolohiia, khirurhiia ta perynatalna medytsyna. 2013; 2 (8): 19-24. (In Russian).
19. Song P., Gao J., Inagaki Y., Kokudo N., Tang W. Rare diseases, orphan drugs, and their regulation in Asia: Current status and future perspectives. Intractable & Rare Dis Res. 2012;1(1):3-9. doi:10.5582/irdr.2012.v1.1.3.
20. Speiser P.W., Azziz R., Baskin L.S., Ghizzoni L. et al. Endocrine Society. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. Available online. 2010; 1: 27-15.
21. Varlamov V.K. Orfannыe zabolevanyia. Novaia nauka: stratehyy y vektorы razvytyia. 2016; №118-2: 7-9.
22. Zubova N.V., Slavko E.A., Tukhanova N.B., Suleimenova Y.M. Klynycheskyi sluchai mukovystsydoza s porazhenyem pecheny. Vestnyk KazNMU. 2016; 4: 11-15. (In Russian).

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.

ORCID and contributionship:
Mariana A. Ryznychuk: 0000-0002-3632-2138 [D]
Vasily P. Pishak: 0000-0002-0637-6936 [F]
Tatiana V. Khmara: 0000-0001-8023-5181 [B]
Nataliia V. Bachuk-Ponych: 0000-0002-3875-5359 [E]
Valentyna N. Pidgirna: 0000-0001-6188-803X [A]
Nina A. Zimahorova: 0000-0003-4551-944X [C]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Mariana A. Ryznychuk
Bukovinian State Medical University
2 Teatralna Square st., 58002 Chernivtsi, Ukraine
tel: +38 050 192 09 53
e-mail: rysnichuk.mariana@gmail.com

Received: 10.12.2019
Accepted: 04.06.2020

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

CASE STUDY

CONGENITAL HEMOLYTIC ANEMIA IN CHILDREN, FEATURES OF THE COURSE AND DIAGNOSIS. THE CLINICAL CASE

DOI: 10.36740/WLek202008138

Veronika M. Dudnyk, Tatyana H. Korol, Hennadii M. Rudenko, Kateryna V. Khromykh, Maryna O. Shalamai
VINNITSA NATIONAL PYROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

We've reported a clinical case of congenital hemolytic anemia which was treated in Vinnitsa Regional Children's Hospital from newborn period until now. We've used complete blood count, biochemichal blood investigation, ultrasound investigation of the abdominal cavity in every hospitalization. Also IFA for TOXO IgG, IgM and G CMV, IgG HSV-6 IgG EBV (EBNA) and IgM EBV, study to hepatitis B and C viruses and HIV were made. There were checked levels of serum iron, ferritin, vitamin B 12 and folic acid in blood serum.

KEY WORDS: congenital hemolytic anemia, children

Wiad Lek. 2020;73(8):1796-1799

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 invascular 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 hemoglobins, 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-

Table I. Amount of hemoglobin and reticulocytes in complete blood count depending on date

Date / Index	June 2016	July 2016	August 2016	September 2016	October 2016
Нв, g/l	50	73	85	53	64
Reticulocytes, ‰	78	210	114	350	235

Table II. Amount of bilirubin depending on date

Date / index	2017			2018		
	April	August	December	April	August	December
General bilirubin, μmol/l	34,2	63,2	89,7	64,4	105,4	96,8
Directed bilirubin, μmol/l	4,3	8,6	6,9	0	4,2	1,8
Indirected bilirubin, μmol/l	26,9	54,6	82,8	64,4	101,2	95,0

eases. So spherocytosis, ovalocytosis, elliptocytosis are more often associated with congenital membranopathies, schistocytes are found in thrombotic microangiopathies, echinocytes - with deficiency of pyruvate kinase, lead poisoning, impaired function of the heart, pituitary gland, equally important laboratory parameters for hemolytic anemia are hemoglobin and reticulocyte levels. Increase level of reticulocytes is one of the main diagnostic criteria for hemolysis, but preference should be given to the bone marrow sensitivity index, which is determined by the formula: absolute patient reticulocyte count × (Nv/normal Nv patient level) [7]. However, in 20-40% of cases observed decrease level of reticulocytes, which is a prognostically unfavorable criterion for the course of the disease. The level of lactate dehydrogenase, indirect bilirubin, haptoglobin, ferritin and direct antiglobulin test are hemolytic indicators that influence the course and determination of hemolytic anemia treatment tactics [13]. Diagnostic methods for clarifying hemolytic anemia also include flow cytometry or the Hem test, which eliminates paroxysmal nocturnal hemoglobinuria. Enzyme activity and molecular study of mutations performed to diagnose enzymatic diseases. Hemoglobin electrophoresis or chromatography is qualitative and quantitative for the elimination of hemoglobinopathies [14].

It is necessary to remember and congenital dyserithropoietic anemia type II in order to carry out differential diagnosis of anemia in young children. The clinical picture of which often includes symptoms of jaundice and splenomegaly. Diserythropoietic anemia belongs to the syndrome of bone marrow and morbidity, which disrupts the differentiation and proliferation of cells of the erythrocyte unit at the level of bone marrow [15]. For diagnostics, it is necessary to take into account the data of hereditary anamnesis, features of clinical course, and laboratory markers of hemolysis, which are often quite specific for differential diagnosis [16]. However, given that hemolytic anemia accounts for a number of heterogeneous diseases, both congenital and acquired, it is not always possible to establish a definitive diagnosis.

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 murmur 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, ampisulbin - 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 0,44; 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

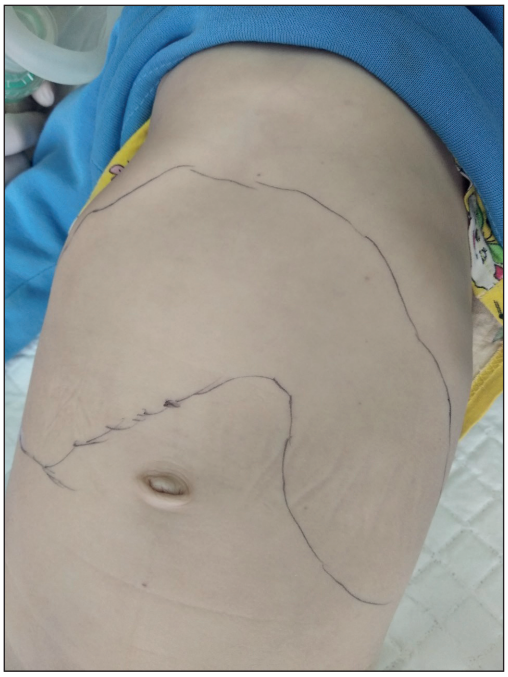

Fig. 1. Enlargement of the spleen - 5-6 cm from the edge of the costal arch

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.

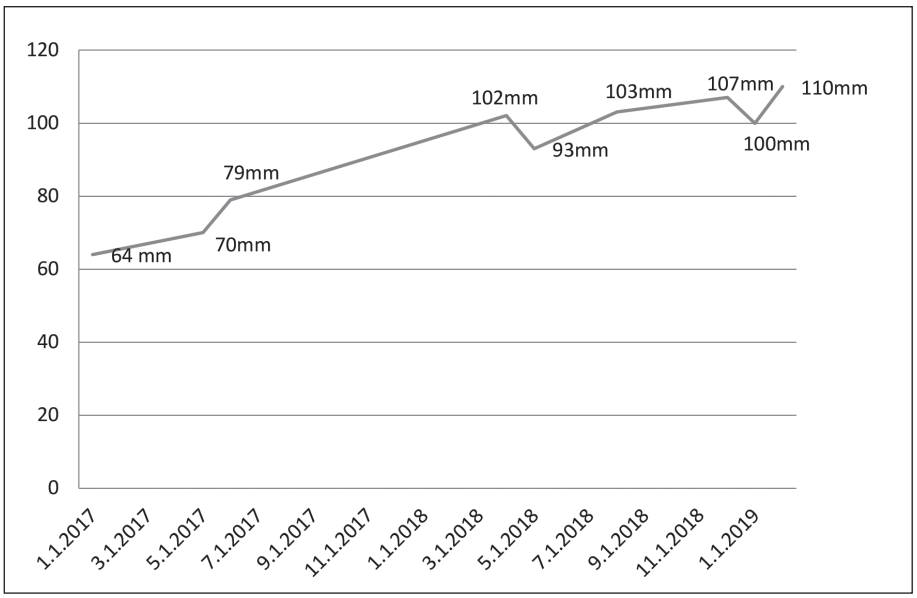

Fig. 2. Dynamic of the spleen size ground on ultrasound data

To exclude fermentopathy, only glucose-6-FDG could be met, which was also within the reference values of 15.27 U/g Nv (6.60 - 17.20).

In order to eliminate 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/u, IgG HSV-6 1,147 opt/u, IgG EBV (EBNA) 0,227 opt/u, IgM EBV 0,003 opt/u , 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.

REFERENCES

1. Micura, E.F. Gemoliticheskie anemii u detej Gomel: GU «RNPC RMiECh», 2017. 20 p.
2. Shih A.W.Y. Haptoglobin testing in hemolysis: measurement and interpretation. American Journal of Hematology. 2014. 4 (89); 443–447.
3. Nagornaya N.V., Vilchevskaya E.V., Bordyugova E.V., Dudchak A.P., et al. Gemoliticheskie anemii u detej. Zdorove rebenka. 2013;8 (51):175-180. (In Russian)
4. Alves M.F.M., Leite J.B.R., Neto M.L.R. et al. A Five-Month-Old Infant With Severe Autoimmune Hemolytic Anemia Treated Without Blood Transfusions. Journal of Medical Cases. 2018;9 (9):284-286.
5. Kuvshinnikov V.A., Shenec S.G. Gemoliticheskie anemii u detej. Ucheb.-metod. posobie. Minsk : BGMU. 2013. 40p. (In Russian)
6. James P. Hemolytic Anemia: Evaluation and Differential Diagnosis. Am Fam Phisician. 2018; 98 (6): 354-361.
7. Barcellini W. Current treatment strategies in autoimmune hemolytic disorders. Expert Review of Hematology. 2015; 5 (8): 681–691.
8. M.R. Pavo Garcia, M. Munoz Dias, M.B. Fernandez. Anemia en la edad pediatrica. Form Act Pediatr Aten Prim. 2016; 9(4): 149-55.
9. Pires F.R., Luglio M., Motta E.H.G. et al. Anemia and thrombocytopenia: attention and care that the pediatrician must have during the service. MOJ Tumor Res. 2018; 1(3): 93–95.
10. Zantek N.D., Koepsell S.A., Tharp D.R., Cohn C.S. The direct antiglobulin test: a critical step in the evaluation of hemolysis. Am J Hematol. 2012; 87(7): 707-9.
11. Junjie F., Hailong H., Wenli Z. et al. Clinical Features and Treatment Outcomes of Childhood Autoimmune Hemolytic Anemia: A Retrospective Analysis of 68 Cases. Journal of Pediatric Hematology/Oncology. 2016; 38 (2): 50-55.
12. Eloy F. R., Miguel A. Cervantes. Diagnostic approach to hemolytic anemias in the adult. Rev Bras Hematol Hemoter. 2015; 37(6): 423-425.
13. Barcellini W., Fattizzo B., Zaninoni A. et al. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: aGIMEMAstudy of 308 patients. Blood. 2014; 124 (19): 2930–2936.
14. Pavo Garcia M.R., Munoz Diaz M., Baro Fernandez M. Anemia en la edad pediatrica. Form Act Pediatr Aten Prim. 2016;9:149-55.
15. Russo R., Gambale A., Langella C., Andolfo I. et al. Retrospective cohort study of 205 cases with congenital dyserythropoietic anemia type II: definition of clinical and molecular spectrum and identification of new diagnostic scores. American Journal of Hematology. 2014; 10 (89): 169-175.
16. Sudipta S.D., Chakrabarty R., Zaman R.U. Das Immunohematological and clinical characterizations of mixed autoimmune hemolytic anemia. Asian Journal of Transfusion Science. 2018; 2 (12): 99-104.

ORCID and contributionship:

Veronika M. Dudnyk: 0000-0003-2164-8204 [A,F]
Tatyana H. Korol: 0000-0002-7240-6056 [B]
Hennadii M. Rudenko: 0000-0003-2799-1900 [C]
Kateryna V. Khromykh: 0000-0001-7241-5190 [D]
Maryna O. Shalamai: 0000-0002-1016-6819 [E]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Kateryna V. Khromykh
Vinnytsia National Pirogov Memorial Medical University
56 Pirogova st., 21018 Vinnytsia, Ukraine
tel: +380634009099
e-mail: kate_khromykh@yahoo.com

Received: 05.10.2019
Accepted: 03.06.2020

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article