CASE REPORT

Fetal–maternal incompatibility in the Rh system. Rh isoimmunization associated with hereditary spherocytosis: case presentation and review of the literature

SIMONA-DANIELA NEAMŢU[1], MARIUS BOGDAN NOVAC[2], ADELA-VALERIA NEAMŢU[3], IULIA DIANA STANCA[4], MIHAIL VIRGIL BOLDEANU[5], ADRIAN GLUHOVSCHI[6], LILIANA STANCA[7], ANDA LORENA DIJMĂRESCU[8], MARIA MAGDALENA MANOLEA[8], MAGDALENA RODICA TRĂISTARU[9], GAROFIŢA-OLIVIA MATEESCU[10], MIRELA ANIŞOARA SIMINEL[11]

[1]Department of Hematology, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania

[2]Department of Anesthesiology and Intensive Care, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

[3]Resident Physician, Department of Anesthesiology and Intensive Care, Prof. Dr. Matei Balş National Institute of Infectious Diseases, Bucharest, Romania

[4]Department of Neurology, University of Medicine and Pharmacy of Craiova, Romania

[5]Department of Immunology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

[6]Department of Obstetrics and Gynecology, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania

[7]Department of Legal Medicine, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

[8]Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania

[9]Department of Medical Rehabilitation, University of Medicine and Pharmacy of Craiova, Romania

[10]Department of Histology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

[11]Department of Neonatology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

Abstract

Next to A and B antigens, agglutinogen D exhibits the highest immunogenicity. Following the transfusion of D-positive red blood cells (RBCs), almost 80% of D-negative recipients develop anti-D antibodies (Abs). Subsequently, anti-D immunization further promotes the synthesis of Abs towards other blood group antigens in or outside the Rh system. The D antigen is also involved in 95% of cases of hemolytic disease of the newborn. Transfusions, hemotherapy, grafts, and obstetric history (abortions, ectopic pregnancy, births) are all risk factors for Rh isoimmunization. In the case of ABO compatibility between mother and fetus, Rh-positive fetal RBCs that have reached the maternal bloodstream are not destroyed by group agglutinins, and Rh antigenic sites are not hidden by the maternal immune system. But a Rh-negative mother with a homozygous Rh-positive husband will certainly have a Rh-positive fetus. As it has an irreversible evolution, the Rh isoimmunization once installed cannot be influenced in the sense of decreasing the Ab titer, therefore, injectable globulin has no effect. A particular case was that of a newborn with Rh system incompatibility associated with hereditary spherocytosis The clinical balance at birth reflects the severe jaundice of the female newborn of 3140 g, gestational age 38/39 weeks, extracted by lower-segment transverse Caesarean section, with a double loop nuchal cord, Apgar score 8. Because the jaundice was severe and atypical (face and upper chest), we considered the possibility of coexistence of hemolytic disease of the newborn by Rh blood group incompatibility associated with hereditary spherocytosis, as it turned out to be true and mentioned. Changes in genes encoding proteins in the structure of the RBC membrane have amplified hemolysis induced by maternal–fetal isoimmunization in the Rh system. Massive hemolysis accentuated by congenital spherocytosis, confirmed later, imposed blood transfusion and dynamic monitoring.

Keywords: isoimmunization, spherocytosis, Rh system, hemolytic anemia.

⊟ Introduction

Hereditary spherocytosis is the most common type of chronic hemolytic anemia caused by a membrane defect found mostly in Caucasians, a condition in which genetic mutations are transmitted autosomal dominantly in 75% of cases. It leads to the synthesis of deformed, spherical red blood cells (RBCs), characterized by low osmotic resistance, splenic sequestration and increased autohemolysis [1, 2].

Maternal–fetal isoimmunizations are pathological conditions in which the pregnant woman is sensitized and produces isoantibodies against fetal blood antigens, the most frequently incriminated being the Rh system. Sensitization during pregnancy occurs when a Rh-negative pregnant woman is exposed to the D antigen present on the fetal RBCs, the Rh being transmitted through the paternal line, in autosomal dominant manner. At first contact with the D antigen, high molecular weight immunoglobulin M

(IgM) antibodies (Abs) are formed, which do not cross the placental barrier and cannot affect the fetus. At a second exposure, the mother develops a very rapid "amnestic" immune response that induces the synthesis of IgG-type Abs, with small molecule weight that cross the placental barrier [3]. Once the process of Ab synthesis is initiated, its evolution is irreversible. Each subsequent pregnancy with Rh-positive fetus, triggers an increasing synthesis of maternal anti-Rh Abs [4, 5]. The association of hereditary spherocytosis with Rh incompatibility amplifies the antigen–Ab conflict in the fetal organism and leads to pathological conditions, such as hemolytic disease of the newborn, fetal–placental anasarca, miscarriage, or even intrauterine death of the fetus.

The peculiarity of our report was the frequency of spherocytes on the blood smear of the newborn associated with an increased titer of anti-D Ig Abs. Severe hemolysis leads to hypoxia and the accumulation of large amount of bilirubin, which can cause jaundice, brain damage, paralysis, blindness, deafness, and intellectual development disorder.

Aim

The aim of this study was to analyze the impact of hemolytic disease on *antepartum* development and *post-partum* and to highlight the need for general prophylaxis measures for hemolytic disease of the fetus and newborn (genetic counseling, phenotyping of ABO and Rh blood group) as prenuptial examination.

⊞ Patients, Materials and Methods

The study included 48 Rh-negative pregnant women with Rh-positive husbands, who were registered at the Filantropia Municipal Hospital, Craiova, Romania, during 2015–2021 and the 48 newborns who underwent clinical examination and paraclinical investigations: blood count, Rh and ABO blood groups, count of reticulocytes, biochemical assays [bilirubin, aspartate aminotransferase (ASAT), alanine amino-transferase (ALAT), creatinine], imagistic explorations.

Hematological, immunological, and biochemical investigations were performed according to the working techniques corresponding to the reagent of the manufacturing company.

The pregnant woman was constantly monitored through medical imaging techniques, and at birth the placenta was sent to the Department of Pathology for the histopathological (HP) examination.

Most pregnant women were medically monitored by constantly determining the titer of anti-Rh Abs, hematological tests [complete blood count (CBC)], coagulation tests [Quick time (QT), international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrin monomer test (FMT), fibrin degradation products (FDPs)], biochemical tests, ultrasound (US) and cardiotocographic examinations. Five of the newborns were diagnosed with hemolytic anemia, and three with severe jaundice due to Rh incompatibility. A particular case was represented by a 25-year-old patient diagnosed with hereditary spherocytosis and Rh iso-immunization.
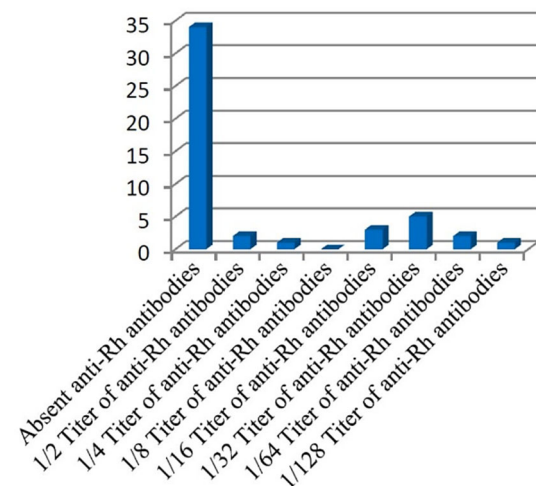
⊞ Case presentation

The massive destruction of fetal RBCs is proportional with the concentration of maternal Abs, which in turn depends on the amount of fetal blood transfused to the mother (Table 1; Figures 1 and 2). Damage to fetal RBCs can range from minimal alteration to massive destruction.
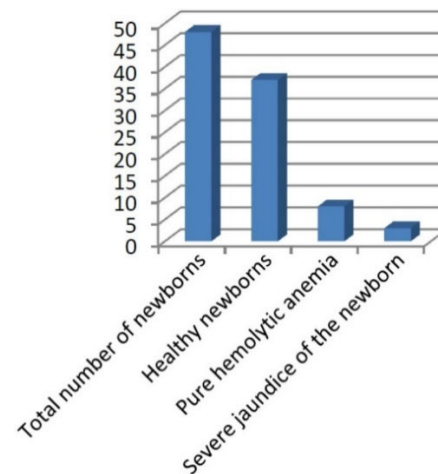
Table 1 – *Titer of anti-Rh Abs in the 48 pregnant women studied*

Titer of anti-Rh Abs	Absent anti-Rh Abs	1/2 Titer	1/4 Titer	1/8 Titer	1/16 Titer	1/32 Titer	1/64 Titer	1/128 Titer
Negative Rh pregnant women	34	2	1	0	3	5	2	1

Abs: Antibodies.

Figure 1 – *Titer of anti-Rh antibodies in pregnant women.*

Figure 2 – *Forms of manifestation of hemolytic disease in newborns.*

We are presenting the case of a newborn whose first clinical manifestation was an acute hemolytic crisis, a newborn with fetal–maternal incompatibility in the Rh system and a family history of hereditary spherocytosis. The mother of the newborn, diagnosed with constitutional hemolytic anemia (hereditary spherocytosis), had been splenectomized since she was 4 years old. Maternal paraclinical investigations confirmed hereditary spherocytosis, A(II) blood group, negative Rh and the presence of anti-Rh Abs in an increased titer of 1/128.

The newborn's blood smear illustrated leukocytosis with neutrophilia and a deviation to the left of the leukocytic formula, an abundance of RBCs precursors in the blood

stream (56 077 erythroblasts/mm³), as well as morphological changes of the RBC series, presence of spherocytes and a 22% accentuated reticulocytosis (Figures 3–6). Laboratory investigations performed initially, *postpartum*, revealed: RBCs 2 040 000/mm³, hemoglobin (Hb) 9.6 g/dL, hematocrit (Ht) 28.1%, mean corpuscular volume (MCV) 137.1 fL, mean corpuscular Hb (MCH) 47.2 pg, mean cell Hb concentration (MCHC) 34.4 g/dL, leukocytes 16 022/mm³, severe erythroblastosis (350 basophilic, polychromatophilic, oxyphilic erythroblasts per 100 leukocytes), ASAT 79 IU/L, ALAT 17 IU/L, increased urobilinogen, total bilirubin 11.48 mg/dL, with predominance of indirect fraction (10.45 mg/dL), A(II) blood group, Rh-negative.

HP examination of the placenta revealed dystrophic villi, fibrinoid necrosis and limestone deposits and areas of recent and old infarction, hemorrhagic dysfunction microfocus and edema (Figures 7–9).

Postnatal immunohematological monitoring of the newborn revealed Rh blood group antigens on fetal RBCs at seven days of *postpartum* therapy. IgG-type anti-Rh Abs,

produced by sensitized maternal lymphocytes, crossed the placental barrier, and covered the fetal RBCs, initially leading to a false negative reaction to identify Rh blood group antigens in the newborn. Compatibility in the ABO system between mother and fetus meant that the fetal RBCs in the maternal bloodstream were not destroyed by group agglutinins, and Rh antigenic sites were not masked for the maternal immune system.

The hepatosplenomegaly secondary to intense hemolysis led to an increase in the volume of the fetal abdomen, with the liver being palpable at 2.5 cm below the right costal rim and the spleen at 2 cm below the left costal rim (Figures 10 and 11).

Due to the extramedullary hematopoiesis, hepatic protein-forming functions decreased (proteinemia 4.6 g/dL), with the alteration of the colloid osmotic pressure, installation of hypoalbuminemia (albumin 2.4 g/dL), extravasation of the plasma and the appearance of edemas. Hemolysis led to the accumulation of large amounts of bilirubin, with the onset of severe jaundice in the newborn.
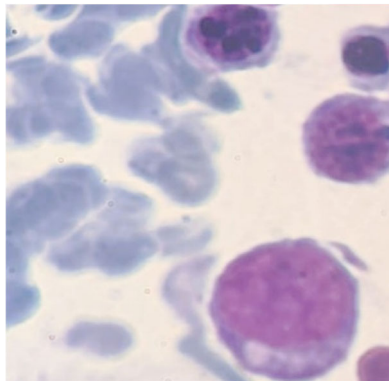

Figure 3 – *Newborn blood smear → abundance of orthochromic, polychromatophilic and basophilic erythroblasts, in the blood stream [May–Grünwald–Giemsa (MGG) staining, ×1000].*
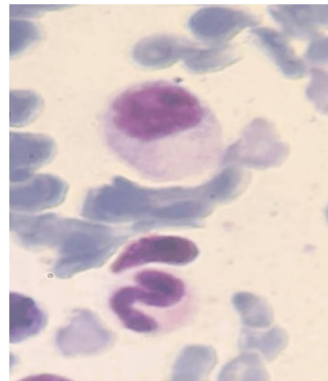

Figure 4 – *Newborn blood smear → leukocytosis with neutrophilia and left shift up to promyelocyte. MGG staining, ×400.*
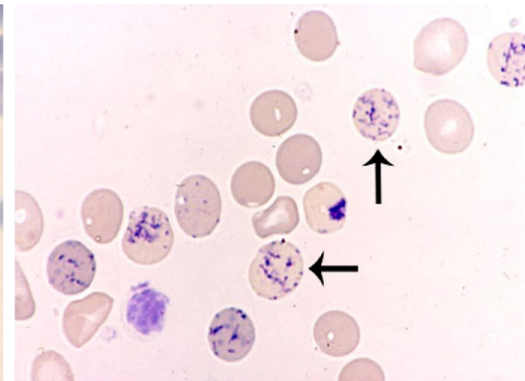
Figure 5 – *Newborn blood smear → 22% hyper-reticulocytosis certifies regenerative anemia, with peripheral production mechanism. Cresyl Blue staining, ×1000.*
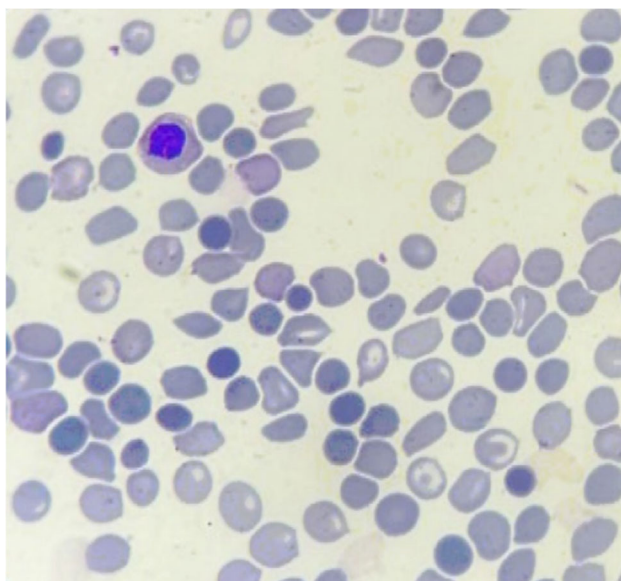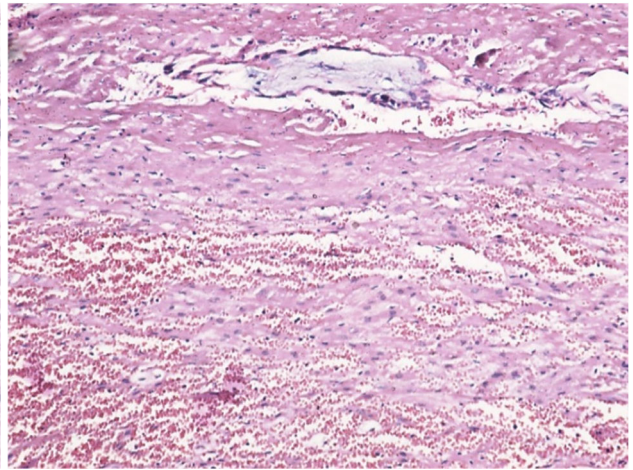

Figure 6 – *Newborn blood smear: polychromatophilic erythroblast and frequent microspherocytes, homogene-ously stained spherical cells with a diameter of 5.5 µm. MGG staining, ×200.*
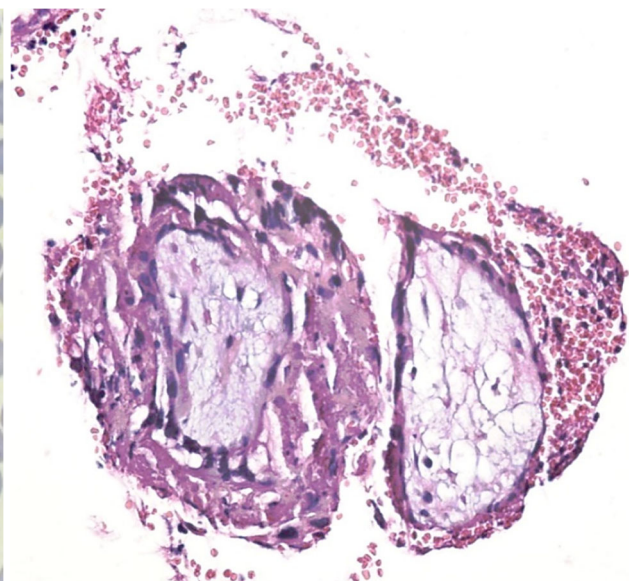
Figure 7 – *Dystrophic placental villi. Hematoxylin–Eosin (HE) staining, ×100.*
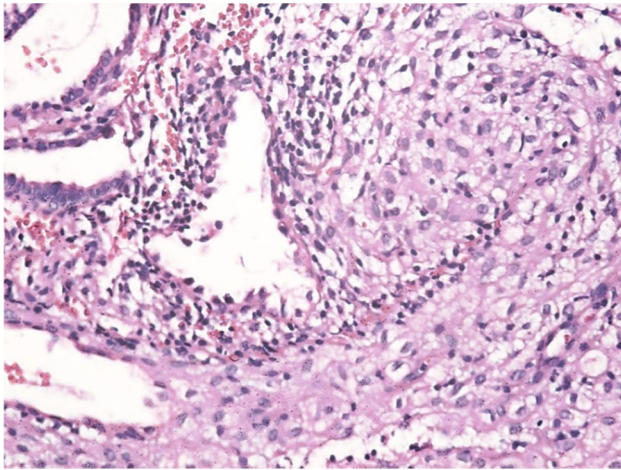
Figure 8 – *Decidua with necrobiosis and inflammatory infiltrate. HE staining, ×200.*

Figure 9 – *Area of hemorrhagic necrosis with "villous capture". HE staining, ×100.*

Figure 10 – *Ultrasound examination: splenomegaly palpable lower pole of the spleen.*

Figure 11 – *Ultrasound examination: hepatomegaly, palpable liver at 2.5 cm below the right costal rim.*

⊟ Discussions

The clinical examination of the newborn reveals that it was born at term: appropriate for gestational age (AGA) with GA 38/39 weeks, female, weighting 3140 g, extracted by Caesarean section with double loop nuchal cord, Apgar score 8/9, incipient jaundice in the face and upper chest, cardio-pulmonary balanced. At birth, free-flow oxygen was delivered, as well as prophylactic phytomenadione, followed by continuous phototherapy using three lamps, preservation of the umbilical stump, intravenous antibiotic therapy (Ampicillin 100 mg/kg/dose), 20% human albumin, enteral feeding with Aptamil 1 first infant milk powder, dynamic monitoring. Congenital diseases, in general, worsen the clinical-biological evolution of the newborn [6].

As mentioned in the medical literature, jaundice is the most common presenting feature of hereditary spherocytosis in neonates, as it happened to our case [7]. Usually, this first symptom of hereditary spherocytosis in neonates is not associated with blood group type incompatibility [8].

Because the jaundice was severe and atypical, we took into consideration the possibility of coexisting condition – ABO hemolytic disease [9], as is turned out and is mentioned.

In addition, it is known that modifications of the genes encoding the RBC membrane proteins (ankyrin, band 3, and spectrin) and the Rh complex happen [8, 10]. In 2018, He *et al.* reviewed the last proposed molecular genetic mechanisms of hereditary spherocytosis and novel mutations in five genes: spectrin alpha, non-erythrocytic 1 (*SPTAN1*), spectrin beta, erythrocytic (*SPTB*), ankyrin 1 (*ANK1*), solute carrier family 4 member 1 (Diego blood group) (*SLC4A1*), and erythrocyte membrane protein band 4.2 (*EPB42*). These genetic aspects could explain the significant heterogeneity of this disease [11]. This is the most common inherited anemia in Northern Europe and Northern America [8]. We didn't performed the genetic profile in our newborn patient.

The particularity in our report was the presence of spherocytes on the blood smear of the neonate, also the typically aspect is less observing of these cells together with sluggish erythropoietic response of neonates often renders the reticulocyte count low relative to the degree of anemia [8].

Hereditary spherocytosis amplifies the degree of hemolytic anemia induced by Rh isoimmunization. Symptoms occur especially towards the end of the pregnancy due to maternal anti-IgG Abs that cross the placental barrier causing RBC

lysis and fetal anemia. The transport of oxygen to the fetal organs is diminished, so the fetus suffers from hypoxia. To make up for the loss of RBCs, the fetus begins to produce more RBCs through bone marrow hyperplasia, a phenomenon that eventually leads to bone deformities of the sternum, skull, or ribs. Accentuated hemolysis exceeds the resources of the bone marrow, and the fetus is forced to resort to other hematopoietic organs (spleen, liver, kidneys) to replace the inefficient erythropoiesis. Hepatosplenomegaly causes an increase in the volume of the fetal abdomen. Due to extramedullary hematopoiesis, liver protein-forming function decreases, and the amount of protein in the blood begins to decrease dramatically, with altered colloid osmotic pressure, resulting in extravasation of plasma with the appearance of edema and fetal ascites [12].

Hemolysis causes the accumulation, in large quantities, of bilirubin, which can cause jaundice with brain damage, paralysis, blindness, deafness and intellectual development disorder. From a clinical point of view, there are several forms of hemolytic disease: pure hemolytic anemia, in which the newborn has low or medium jaundice, with a short duration, severe jaundice of the newborn, manifested *postpartum* due to excess bilirubin, caused by excessive lysis of the RBCs and intrauterine fetal–placental anasarca. The latter is a consequence of severe fetal anemia in which the fetus has ascites, hepatomegaly, generalized edema, hydramnios. In the absence of birth, it leads to fetal death *in utero* [13].

Rh isoimmunizations diagnosed by titer of anti-D Abs in the maternal blood, indicated by the indirect Coombs test, known to be the method with the highest sensitivity. Isoimmunization is pre-existing to the pregnancy when the Abs are detected in the first 12 weeks of pregnancy. If anti-Rh Abs appear after 26–28 weeks of gestation, isoimmunization occurs during pregnancy. In 1/8 titrated Rh isoimmunizations, Ab dosing is repeated at 4-week intervals. Rh isoimmunizations with 1/64 titer generate legal termination of pregnancy, the fetal consequences being particularly serious. If the pregnancy evolves with constant titers, US and amniocentesis are performed to assess the health of the fetus [14, 15].

A rapid increase in the concentration of Abs during a short period of time indicates the risk of fetal damage. In advanced pregnancy, a sudden decrease in titer may be due to either the immunosuppressive effect of pregnancy or the massive transfer of Abs into the fetal circulation and their attachment to the fetal RBCs, requiring immediate therapeutic measures.

The US examination gives information on the thickness of the placenta, the diameter of the umbilical vein, the fetal abdominal circumference and indirectly on the size of the hepatosplenic block. The Doppler US allows the assessment of the placental resistance index, which decreases in severe forms leading to an increase in the blood flow in the umbilical vein. The measurement of maximum velocity of the middle cerebral artery is the most sensitive predictive parameter of fetal anemia, and Doppler US evaluations in cases with isoimmunization to detect fetal anemia should be done weekly, starting with the GA of 28 weeks. The appearance of a sinusoidal pathway during the cardiotocographic examination is a sign of fetal anemia with severe insufficiency.

Amniotic fluid analysis can reveal Hb degradation products and coagulation disorders. Amniocentesis is indicated at an Ab titer of 1/64 or higher in isoimmunizations developed during the evolution of the respective pregnancy, at an Ab titer of 1/32 or higher in pre-existing isoimmunization, in case of pathological evolution of pregnancy (preeclampsia, eclampsia, hydramnios) or if specified in the history of intra-uterine fetal death, severe neonatal jaundice, exsanguino-transfusion or placental anasarca. Cordocentesis allows the evaluation of the degree of anemia by determining the fetal Hb value, which can also become a therapeutic gesture.

The therapeutic principles vary depending on the titer of anti-Rh Abs. When Ab titers are low, fetal US monitoring and Doppler US determination of fetal blood flow on the middle cerebral artery are sufficient. When the Ab titer is increasing, the fetal health condition should be intensively monitored by US, cardiotocography, amniocentesis and cordocentesis. The degree of fetal anemia must be assessed and corrected in isoimmunized patients until the maturity of the fetal lungs is reached so that birth is possible without risks [16–18].

Plasmapheresis is indicated in women with severe isoimmunization, with a high Ab titer, before 20 weeks of gestation to decrease the concentration of Abs in the maternal blood less than 1 μg/mL. In critical cases, premature birth is triggered if the fetus is over 32–34 weeks old and intensive *postpartum* therapy is practiced, or intrauterine treatments are administered if the GA is less than 32–34 weeks. Fetal transfusion *in utero* can be applied starting with week 22 of pregnancy based on the biochemical results of the amniotic fluid and of fetal Hb.

Determining the Rh factor, as a prenuptial examination, to prevent fetal–maternal immune conflict, maintaining the first pregnancy in Rh-negative women with Rh-positive husbands, spacing pregnancies at least three years apart to avoid amnestic immunological reactions, avoiding iso-immunization of Rh-negative women by transfusions of incompatible blood or graft are general prophylaxis measures.

The special prophylaxis of fetal–maternal incompatibility in the Rh system consists of the administration of 300 μg of anti-D Ig to Rh-negative patients non-immunized with Rh-positive husbands, between 28–30 weeks of gestation, and a new administration in the first 72 hours after birth only if the newborn is Rh-positive, the life of these Abs being about 12 weeks [19].

In the last two years, it was mentioned that although anti-D alloimmunization is the most common cause of hemolytic disease of the fetus and newborn, more than 50 anti-RBC alloantibodies are involved, and a part of these non-anti-D Abs increased after the implementation of pre- and postnatal anti-D prophylaxis.

The transfusion need and perinatal outcome are related to the type of Ab, with RhD isoimmunization being the most frequent and most serious during pregnancy [20].

IgD should also be administered in case of an incompatible transfusion in the Rh system, in case of an abortion, of an ectopic pregnancy or after the practice of amniocentesis in non-immunized Rh-negative women [21, 22].

Hereditary spherocytosis is one of the congenital hemolytic anemias – a heterogeneous group of rare hereditary conditions characterized by reduced life span and premature removal

of the RBCs from the circulation. Although few data are reported on the role of the immune system in these sanguine disorders, several immune-mediated mechanisms are certainly involved in the pathogenesis of these rare diseases, namely naturally occurring autoantibodies and this aspect may explain the complex pathogenic pattern in newborn [23].

⊡ Conclusions

Fetal–maternal isoimmunization in the Rh system has amplified hemolysis due to the mutation of various genes responsible for encoding proteins involved in the structure of the RBC membrane (beta-spectrin, alpha-spectrin, ankyrin, protein 4.2) leading to the synthesis of deformed, spherical RBCs, which have low resistance to deformation, increased membrane permeability to sodium and water, that are being recognized by the spleen and passively destroyed. The obstruction of D antigenic sites on fetal RBCs by maternal Abs that have crossed the placental barrier causes hemolytic anemia in the newborn and may lead to false negative reactions to identify Rh blood group antigens in the newborn in the first few days after birth, therefore postnatal immunohematological monitoring in dynamic is required.

Conflict of interests
The authors declare that they have no conflict of interests.

Authors' contribution
Simona-Daniela Neamțu and Marius Bogdan Novac equally contributed to the manuscript.

References

[1] American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 75: Management of alloimmunization during pregnancy. Obstet Gynecol, 2006, 108(2):457–464. https://doi.org/10.1097/00006250-200608000-00044 PMID: 16880320

[2] Liu KL, Li N, Li BJ, Peng JY, Shu XW, Yu YY. [Clinical significance of anti-D IgG screening and titer detection in 286 RhD negative pregnant women]. Zhongguo Dang Dai Er Ke Za Zhi (Chin J Contemp Pediatr), 2009, 11(3):185–187. PMID: 19292952

[3] Zimmerman R, Carpenter RJ Jr, Durig P, Mari G. Longitudinal measurement of peak systolic velocity in the fetal middle cerebral artery for monitoring pregnancies complicated by red cell alloimmunisation: a prospective multicentre trial with intention-to-treat. BJOG, 2002, 109(7):746–752. https://doi.org/10.1111/j.1471-0528.2002.01314.x PMID: 12135209

[4] Moise KJ Jr. Diagnosing hemolytic disease of the fetus – time to put the needles away? N Engl J Med, 2006, 355(2):192–194. https://doi.org/10.1056/NEJMe068071 PMID: 16837685

[5] Neamțu SD, Novac MB, Fortofoiu M, Fortofoiu MC, Siminel M. Maternal–fetal incompatibility isoimmunization in Rh system. Proceedings of the 16th International Multidisciplinary Scientific GeoConference (SGEM 2016), Albena, Bulgaria, 30 June–6 July 2016. Book 6, Volume 1: Nano, Bio and Green Technologies for Sustainable Future, Section 25: Advances in Biotechnology, Curran Associates, Inc., New York, USA, 2016, 637–644. https://doi.org/10.5593/SGEM2016/B61/S25.084 http://toc.proceedings.com/31816webtoc.pdf

[6] Neamțu S, Găman G, Stanca L, Buzatu I, Dijmărescu L, Manolea M. The contribution of laboratory investigations in

diagnosis of congenital infections. Rom J Morphol Embryol, 2011, 52(1 Suppl):481–484. PMID: 21424097

[7] Christensen RD, Henry E. Hereditary spherocytosis in neonates with hyperbilirubinemia. Pediatrics, 2010, 125(1):120–125. https://doi.org/10.1542/peds.2009-0864 PMID: 19948573

[8] Ciepiela O. Old and new insights into the diagnosis of hereditary spherocytosis. Ann Transl Med, 2018, 6(17):339. https://doi.org/10.21037/atm.2018.07.35 PMID: 30306078 PMCID: PMC6174190

[9] Christensen RD, Yaish HM, Gallagher PG. A pediatrician's practical guide to diagnosing and treating hereditary spherocytosis in neonates. Pediatrics, 2015, 135(6):1107–1114. https://doi.org/10.1542/peds.2014-3516 PMID: 26009624 PMCID: PMC4444801

[10] Manciu S, Matei E, Trandafir B. Hereditary spherocytosis – diagnosis, surgical treatment and outcomes. A literature review. Chirurgia (Bucharest), 2017, 112(2):110–116. https://doi.org/10.21614/chirurgia.112.2.110 PMID: 28463670

[11] He BJ, Liao L, Deng ZF, Tao YF, Xu YC, Lin FQ. Molecular genetic mechanisms of hereditary spherocytosis: current perspectives. Acta Haematol, 2018, 139(1):60–66. https://doi.org/10.1159/000486229 PMID: 29402830

[12] Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, Dorman KF, Ludomirsky A, Gonzalez R, Gomez R, Oz U, Detti L, Copel JA, Bahado-Singh R, Berry S, Martinez-Poyer J, Blackwell SC. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. N Engl J Med, 2000, 342(1):9–14. https://doi.org/10.1056/NEJM200001063420102 PMID: 10620643

[13] Oepkes D, Seaward PG, Vandenbussche FP, Windrim R, Kingdom J, Beyene J, Kanhai HHH, Ohlsson A, Ryan G; DIAMOND Study Group. Doppler ultrasonography *versus* amniocentesis to predict fetal anemia. N Engl J Med, 2006, 355(2):156–164. https://doi.org/10.1056/NEJMoa052855 PMID: 16837679

[14] Gottvall T, Hildén JO. Concentration of anti-D antibodies in Rh(D) alloimmunized pregnant women, as a predictor of anemia and/or hyperbilirubinemia in their newborn infants. Acta Obstet Gynecol Scand, 1997, 76(8):733–738. https://doi.org/10.3109/00016349709024338 PMID: 9348249

[15] Roberts AB, Mitchell JM, Lake Y, Pattison NS. Ultrasonographic surveillance in red blood cell alloimmunization. Am J Obstet Gynecol, 2001, 184(6):1251–1255. https://doi.org/10.1067/mob.2001.112975 PMID: 11349197

[16] Detti L, Oz U, Guney I, Ferguson JE, Bahado-Singh RO, Mari G; Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. Doppler ultrasound velocimetry for timing the second intrauterine transfusion in fetuses with anemia from red cell alloimmunization. Am J Obstet Gynecol, 2001, 185(5):1048–1051. https://doi.org/10.1067/mob.2001.118161 PMID: 11717631

[17] Dukler D, Oepkes D, Seaward G, Windrim R, Ryan G. Noninvasive tests to predict fetal anemia: a study comparing Doppler and ultrasound parameters. Am J Obstet Gynecol, 2003, 188(5):1310–1314. https://doi.org/10.1067/mob.2003.265 PMID: 12748504

[18] Scheier M, Hernandez-Andrade E, Carmo A, Dezerega V, Nicolaides KH. Prediction of fetal anemia in rhesus disease by measurement of fetal middle cerebral artery peak systolic velocity. Ultrasound Obstet Gynecol, 2004, 23(5):432–436. https://doi.org/10.1002/uog.1010 PMID: 15133790

[19] Liumbruno GM, D'Alessandro A, Rea F, Piccinini V, Catalano L, Calizzani G, Pupella S, Grazzini G. The role of antenatal immunoprophylaxis in the prevention of maternal–foetal anti-Rh(D) alloimmunisation. Blood Transfus, 2010, 8(1):8–16. https://doi.org/10.2450/2009.0108-09 PMID: 20104273 PMCID: PMC2809506

[20] Sánchez-Durán MÁ, Higueras MT, Halajdian-Madrid C, Avilés García M, Bernabeu-García A, Maiz N, Nogués N, Carreras E. Management and outcome of pregnancies in women with red cell isoimmunization: a 15-year observational study from a tertiary care university hospital. BMC Pregnancy Childbirth, 2019, 19(1):356. https://doi.org/10.1186/s12884-019-2525-y PMID: 31615430 PMCID: PMC6794826

[21] Onesimo R, Rizzo D, Ruggiero A, Valentini P. Intravenous Immunoglobulin therapy for anti-E hemolytic disease in the newborn. J Matern Fetal Neonatal Med, 2010, 23(9):1059–1061. https://doi.org/10.3109/14767050903544751 PMID: 20092394

[22] Gaines AR. Disseminated intravascular coagulation associated with acute hemoglobinemia or hemoglobinuria following Rh(0)(D) immune globulin intravenous administration for immune thrombocytopenic purpura. Blood, 2005, 106(5):1532–1537. https://doi.org/10.1182/blood-2004-11-4303 PMID: 15878975

[23] Zaninoni A, Fermo E, Vercellati C, Marcello AP, Barcellini W, Bianchi P. Congenital hemolytic anemias: is there a role for the immune system? Front Immunol, 2020, 11:1309. https://doi.org/10.3389/fimmu.2020.01309 PMID: 32655575 PMCID: PMC7324678

Corresponding authors
Adela-Valeria Neamţu, MD, Resident Physician, Department of Anesthesiology and Intensive Care, Prof. Dr. Matei Balş National Institute of Infectious Diseases, 1 Calistrat Grozovici Street, Sector 2, 021105 Bucharest, Romania; Phone +40749–792 417, e-mail: nav29.adela@yahoo.com

Liliana Stanca, Lecturer, MD, PhD, Department of Legal Medicine, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40740–197 898, e-mail: lillystanca@yahoo.com