IMMANUEL KANT BALTIC FEDERAL UNIVERSITY

RH ALLOIMMUNIZATION IN PREGNANCY: COMPREHENSIVE STRATEGIES FOR THE DIAGNOSIS AND MANAGEMENT OF HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN

Training manual

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R51 **Rh Isoimmunization in Pregnancy, Hemolytic Disease of the Fetus and Newborn: Management, Diagnosis, Treatment** : training manual / compilers A. I. Pashov, N. N. Nikishov, A. S. Turdieva, A.Sh. Turdiev. — Kaliningrad : Immanuel Kant Baltic Federal University Press, 2026. — 56 p.
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This textbook for the discipline "Obstetrics and Gynecology" complies with the requirements of the Federal State Educational Standard of Higher Education (2020) for specialty 31.05.01 General Medicine. The present textbook covers issues of Rh isoimmunization in pregnancy, as well as hemolytic disease of the fetus and newborn. Includes figures, tables, test questions.

It is intended for students in their 4th—6th years of study in specialty 31.05.01 General Medicine.

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ОГЛАВЛЕНИЕ

PREFACE

Despite significant advances in perinatal medicine, the problem of hemolytic disease of the fetus and newborn in the context of immunoconflict pregnancy cannot be considered completely resolved in our country [2; 3; 14; 15]. Hemolytic disease of the fetus is diagnosed in approximately 0.6% of newborns in the Russian Federation, and the incidence of Rh isoimmunization has not yet shown a significant downward trend in recent years [14; 15].

Perinatal mortality rates due to hemolytic disease of the fetus remain high, ranging from 15 to 16‰ [14; 15; 16]. A significant reduction in perinatal morbidity and mortality from hemolytic disease of the fetus is impossible without organizing measures for the timely and universal prevention of Rh isoimmunization during pregnancy and in the early postpartum period at the population level.

Furthermore, advances in molecular genetic technologies have now made it possible to non-invasively determine the fetal Rh genotype in an Rh-negative mother as early as the end of the first trimester of pregnancy through prenatal testing of cell-free fetal DNA in maternal blood samples, with a sensitivity and specificity of 98—100%. The possibility of non-invasive determination of the fetal Rh genotype in Rh-negative pregnant women allows for a reduction in pregnancy management costs, avoidance of multiple screening tests for Rh antibodies, and ensures the preventive use of anti-Rh immunoprophylaxis only in cases of a Rh-positive fetal genotype. If determining the fetal Rh genotype is not possible, the pregnancy in an Rh-negative woman should be managed as if carrying a Rh-positive fetus [14; 15].

In Rh-isoimmunized women, determining the fetal Rh-D gene avoids unnecessary invasive diagnostic and therapeutic procedures if the fetal blood type is Rh-negative. In cases of a Rh-positive fe-

tus, the method allows for early assessment of the risk of developing hemolytic disease of the fetus and timely initiation of diagnostic measures.

The use of preimplantation genetic testing in IVF programs when the father has a heterozygous genotype for the Rh factor (RhD+/RhD–) enables families who have experienced fetal or newborn loss due to hemolytic disease to perform selective transfer of embryos with a homozygous Rh-negative genotype into the uterine cavity, thereby preventing the potential development of hemolytic disease in these fetuses.

It is important to note that for the healthcare system, the costs associated with implementing the prevention of Rh isoimmunization are significantly lower compared to the costs of treating hemolytic disease of the fetus and newborn [14; 15].

Part I

RHESUS (RH) ISOIMMUNIZATION IN PREGNANCY. HEMOLYTIC DISEASE OF THE FETUS

Definition

Rh isoimmunization is the presence of IgG antibodies (anti-Rh(D) antibodies) in the maternal blood. It is a manifestation of a secondary immune response in sensitized women resulting from incompatibility between maternal and fetal blood based on antigens of the Rhesus system (synonyms: Rh isoimmunization, Rh conflict, Rh sensitization, Rh alloimmunization).

Hemolytic disease of the fetus (HDF) is a condition characterized by the hemolysis of Rh(D)-positive fetal erythrocytes under the influence of maternal anti-Rh(D) antibodies that cross the placental barrier into the fetal bloodstream. This occurs due to incompatibility between maternal and fetal blood based on the Rhesus system and manifests as the development of anemia and an increased number of erythroblasts (synonyms: erythroblastosis fetalis, hemolytic jaundice).

Etiology and Pathogenesis

The Rhesus (Rh) factor is a system of human allogeneic erythrocyte antigens. It is one of over 40 blood group systems recognized by the International Society of Blood Transfusion (ISBT) and is the most important system after the AB0 system.

The Rhesus factor system consists of blood groups determined by 55 antigens, among which the antigens with the highest immunogenic properties are of the greatest practical medical importance:

D, C, c, E, e. Depending on the individual, the antigen Rho(D) of the Rhesus factor system may be present or absent on the surface of erythrocytes. This antigen is the most immunogenic of the Rhesus system blood group antigens. Its presence is denoted as Rh+ for Rh-positive blood (possessing the Rho(D) antigen) (Fig. 1), and its absence is denoted as Rh– for Rh-negative blood (lacking the Rho(D) antigen) (Fig. 2).

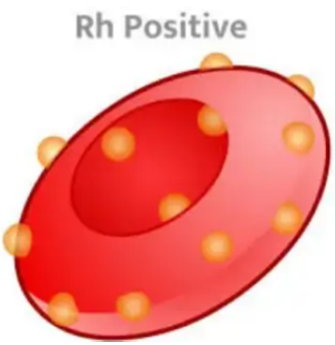


Fig. 1. Rh-positive blood

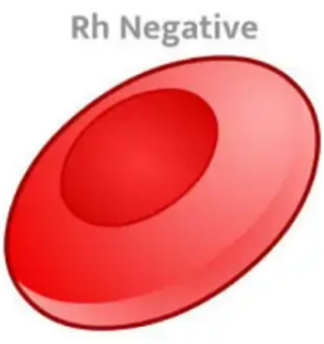


Fig. 2. Rh-negative blood

Inheritance of the D Antigen. Rh conflict primarily develops due to incompatibility between maternal and fetal blood regarding the RhD antigen.

Inheritance patterns for any trait are categorized as homozygous or heterozygous: DD — homozygous; Dd — heterozygous; dd — homozygous (where D is the dominant gene and d is the recessive gene). When considering the transmission of hereditary material, it is important to account for the father's Rh factor homozygosity or heterozygosity.

If the father of the unborn child is homozygous (DD), which is observed in 40—45 % of all Rh-positive men, the dominant D gene is always transmitted to the fetus. Consequently, an Rh-negative woman (dd) will have an Rh-positive fetus in 100 % of such cases (Fig. 3).

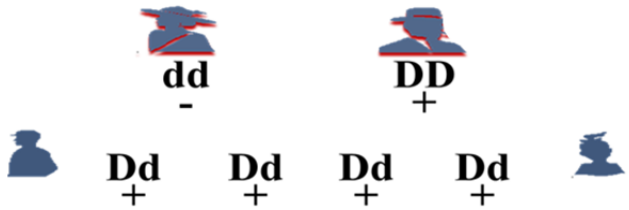


Fig. 3. Transmission of genetic material from a homozygous (DD) father

If the father is heterozygous (Dd), which applies to approximately 55—60 % of all Rh-positive men, the fetus may be Rh-positive in 50 % of cases, as inheritance of either the dominant or the recessive gene is possible (Fig. 4).

Fig. 4. Transmission of genetic material from a heterozygous (Dd) father

A woman with Rh-negative blood becomes sensitized either during pregnancy when the fetal Rh(D) antigen, inherited from the biological father, enters her bloodstream, or outside of pregnancy through the transfusion of Rh(D)-positive donor blood components.

During pregnancy, isoimmunization occurs as a result of feto-maternal transplacental microtransfusions, the frequency of which increases with gestational age. Specifically, fetal erythrocytes cross the placental barrier into the maternal bloodstream during the first trimester in 5—7 % of women, during the second trimester in 15—16 %, and during the third trimester in 29—30 % of women.

The first stage of the maternal immune response is the production of IgM antibodies, which have a high molecular weight and do not cross the placental barrier into the fetal bloodstream. The for-

mation of a primary immune response typically requires at least 6—8 weeks. Therefore, the development of hemolytic disease of the fetus (HDF) during a first pregnancy is extremely rare. HDF in a first pregnancy may result from pre-existing isoimmunization, for example, due to a history of an Rh-negative woman receiving Rh-positive blood components.

The frequency of primary isoimmunization during a first Rh-incompatible pregnancy is less than 1 %, and, in the absence of its prevention by administering anti-Rh immunoglobulin postpartum, it reaches 10 %. The development of HDF due to the Rh factor generally occurs in subsequent pregnancies.

The subsequent stages of isoimmunization involve the formation of IgG antibodies, which have a low molecular weight and freely cross the placental barrier into the fetal bloodstream. This includes IgG subclasses G1 and G3, which actively interact with Fc receptors (FcR) on lymphocytes and macrophages, playing an important role in the hemolysis of fetal erythrocytes (Fig. 5).

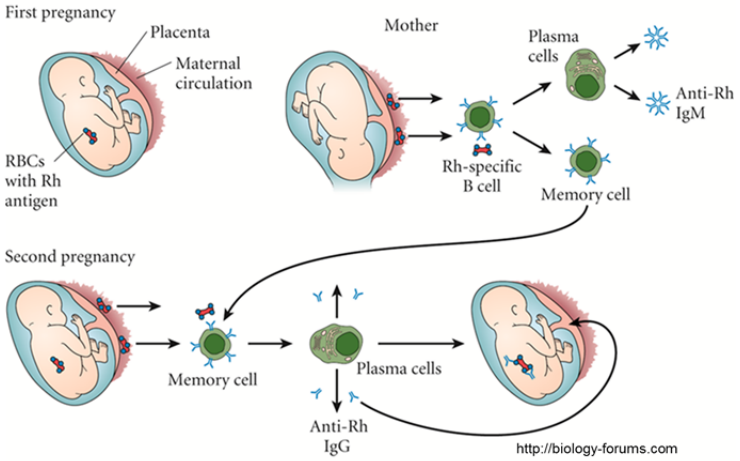


Fig. 5. Pathogenesis of Rh Immunization During the First and Subsequent Pregnancies

Note: maternal circulation — uteroplacental circulation; RBCs with Rh antigen — Rh(+) erythrocyte; anti-Rh IgM — anti-Rh IgM antibody; anti-Rh IgG — anti-Rh IgG antibody.

As a result of hemolysis, the fetus develops anemia, which leads to foci of extramedullary hematopoiesis and hepatosplenomegaly. Due to the "overload" of liver cells with iron and globin breakdown products, its protein-synthetic function is impaired. This leads to hypoproteinemia, hypoalbuminemia, and subsequently to fibrosis and hypertension in the portal and umbilical veins, along with increased vascular wall permeability. Against the background of progressive anemia, hypoxemia develops, causing a hyperdynamic type of circulation in the fetus. This gradually progresses to heart failure and portal hypertension, which further contributes to the enlargement of the liver and the development of hydrops fetalis (anasarca). This is how the most severe — edematous — form of HDF develops.

In the absence of intrauterine treatment, severe hemolytic disease can lead to antenatal fetal death. The relatively milder icteric form of HDF at birth is caused by a later onset of fetal erythrocyte hemolysis shortly before delivery or immediately after birth.

Epidemiology

In the Russian Federation, hemolytic disease is diagnosed in approximately 0.6—1.0% of newborns, and the incidence of Rh isoimmunization has not yet shown a substantial downward trend in recent years.

Perinatal mortality from HDF is 0.037‰. The case fatality rate for HDF in Russia is about 0.22%. The incidence of bilirubin encephalopathy worldwide ranges from 0.4 to 2.7 per 100,000 newborns.

Classification

Based on the nature of the immunological conflict between maternal and fetal blood:

- incompatibility regarding the Rh factor;
- incompatibility regarding the ABO system;
- incompatibility regarding other erythrocyte antigens.

Forms of HDF based on anemia severity and presence of fetal hydrops:

- moderate anemia;
- severe anemia;
- severe anemia with fetal hydrops.

Forms of HDF based on fetal hemoglobin / hematocrit levels:

- mild anemia — hemoglobin deficit within 20 g/L compared to the median for the gestational age (Hb < 0.84 MoM);
- moderate-to-severe anemia — deficit within 20—70 g/L (Hb < 0.65 MoM);
- severe anemia — deficit greater than 70 g/L (Hb < 0.55 MoM).

Diagnosis of Hemolytic Disease of the Fetus

Complaints and Medical History. There are no specific complaints in pregnant women with Rh isoimmunization during the early stages of HDF development.

Collection and analysis of anamnestic risk factors for Rh isoimmunization.

Establish the ABO blood group, Rh factor of the pregnant woman, as well as the blood group and Rh factor of the biological father. Determine the presence or absence of Rh antibodies.

Ascertain a history of blood component transfusions administered to the Rh-negative woman without considering Rh compatibility.

Analyze data on the number of pregnancies, their course, and outcomes (deliveries, induced abortions, miscarriages, missed abortions, ectopic pregnancies) in women with Rh-negative blood who were pregnant by an Rh-positive partner. Inquire about any history of Rh isoimmunization prophylaxis via administration of anti-Rh immunoglobulin (note the timing and dosage).

Obtain anamnestic data regarding the birth of previous children with signs of HDF, methods of treatment, gestational age at delivery, and the severity of the disease in the newborns.

Identify complications during the current pregnancy (bleeding during pregnancy, intrauterine fetal death in the current pregnancy, abdominal trauma, and the performance of invasive diagnostic or therapeutic interventions such as chorionic villus sampling, amniocentesis, cordocentesis, cerclage, fetal reduction in multifetal pregnancy, external cephalic version for breech presentation). Inquire about any history of Rh isoimmunization prophylaxis via administration of anti-Rh immunoglobulin (note the timing and dosage).

Clinical Presentation

With the development of severe anemia and the edematous form of HDF, the pregnant woman may note a decrease in the intensity of fetal movements, reflecting reduced fetal motor activity due to developing heart failure. During examination in the second and third trimesters, clinical signs of polyhydramnios may be detected.

The primary role in diagnosing HDF is assigned to laboratory and functional investigative methods, which determine the severity of the fetal condition.

Physical Examination

Is conducted in accordance with the examination of pregnant women, as stipulated by: order of the Ministry of Health of the Russian Federation dated October 20, 2020, No. 1130n "On Approval of the Procedure for Providing Medical Care in the Field of 'Obstetrics and Gynecology (excluding the use of assisted reproductive technologies)'."

Laboratory Diagnostics of Rh Isoimmunization.

During the initial prenatal registration, the pregnant woman's ABO blood group and Rh factor are determined.

If an Rh-negative patient is identified, the titer of anti-Rh antibodies in her blood is determined. The blood group and Rh factor of the biological father are also established.

Frequency of Anti-Rh Antibody Titer Testing: Testing is performed at the first visit, then at 18—20 weeks, and again at 28 weeks of gestation. More frequent testing for anti-Rh antibodies is not recommended. This is because trace levels of anti-Rh antibody titers may be detectable for up to 12 weeks after the antenatal prophylactic administration of anti-Rh(D) immunoglobulin, which can lead to false-positive results and makes routine screening during this period non-informative.

The diagnosis of Rh isoimmunization is based on the detection of anti-Rh antibodies in the blood, and the severity of isoimmunization is assessed based on the magnitude of the anti-Rh antibody titer.

However, the detection and titer value of antibodies do not reliably establish the presence or severity of hemolytic disease of the fetus, especially when the father has a heterozygous Rh genotype. If anti-Rh antibodies are detected, the pregnant woman should be referred for consultation to a Level III medical facility (Perinatal Center) for further specialized management, in accordance with the established patient care pathways in obstetrics and gynecology.

Non-Invasive Determination of Fetal Rh Genotype via Analysis of Cell-Free DNA in Maternal Blood.

This is performed at the end of the first trimester of pregnancy through prenatal testing of cell-free fetal DNA in maternal blood (the method's sensitivity and specificity are 98—100%).

Non-invasive determination of the fetal Rh genotype in non-immunized Rh-negative pregnant women allows for a reduction in pregnancy management costs and ensures that prophylactic anti-Rh immunoprophylaxis is administered only when the fetus has an Rh-positive genotype. If determination of the fetal Rh genotype is not possible, the pregnancy should be managed as if carrying an Rh-positive fetus.

In Rh-immunized women, determining the fetal Rh-D genotype and identifying a Rh-negative fetus allows for the avoidance of unnecessary invasive diagnostic and therapeutic interventions.

In cases where a Rh-positive fetus is identified, it provides an opportunity to assess the risk of developing hemolytic disease of the fetus early in pregnancy and to initiate timely diagnostic measures.

Prenatal Diagnosis of Hemolytic Disease of the Fetus by Determining the Peak Systolic Velocity in the Middle Cerebral Artery (MCA-PSV).

This assessment is performed in cases of maternal Rh isoimmunization, starting from 18—19 weeks of gestation. After the 35th week of pregnancy, the sensitivity and specificity of this non-invasive test decrease somewhat, and a comprehensive assessment of fetal status should additionally include ultrasound fetometry and cardiotocography (CTG).

Modern prenatal diagnosis of hemolytic disease of the fetus is based on the non-invasive assessment of the severity of fetal anemia using the peak systolic velocity in the middle cerebral artery (MCA-PSV). From the late second trimester and throughout the third trimester, this measurement shows a strong correlation with fetal hematocrit and hemoglobin levels.

An increase in the peak systolic velocity in the fetal middle cerebral artery above the gestational age-specific threshold with high sensitivity and specificity indicates the development of a hyperdynamic circulation in the fetus, which is characteristic of severe anemia requiring intervention during pregnancy.

The use of the fetal MCA-PSV has significantly reduced the need for invasive diagnostic procedures (cordocentesis) to obtain fetal blood for analysis.

Procedure.

MCA-PSV measurement is performed on an outpatient basis, in the consulting department of a regional center capable of performing diagnostic and therapeutic intrauterine invasive interventions. It involves performing a series of consecutive Doppler measurements of the MCA-PSV.

Interpretation.

An increased MCA-PSV for the corresponding gestational age, with high sensitivity and specificity, indicates the development of fetal anemia of varying severity. An MCA-PSV less than or equal

to 1.29 MoM indicates the absence of fetal anemia or a mild form of the disease. An MCA-PSV between 1.29 MoM and 1.5 MoM suggests moderate anemia. тAn MCA-PSV greater than 1.5 MoM indicates severe fetal anemia.

The sensitivity of MCA-PSV for predicting moderate fetal anemia (hemoglobin concentration <0.65 MoM) and severe anemia (hemoglobin concentration <0.55 MoM) in the absence of fetal hydrops reaches 100%, with a false-positive rate of 12%. The use of fetal MCA-PSV has significantly reduced the need for diagnostic cordocentesis.

Other Ultrasound Assessments.

Prenatal diagnosis of hemolytic disease of the fetus using ultrasound to measure fetometric parameters (hepatomegaly, splenomegaly, placental thickness) or Doppler parameters of blood flow (in the fetal aorta, umbilical vein, and umbilical arteries) has low accuracy in determining the severity of hemolytic disease and is currently not used for this purpose.

The sonographic detection of ascites and hydrops fetalis (a combination of hydropericardium, hydrothorax, and subcutaneous edema of the fetal head, trunk, and limbs) represents a late finding indicative of an extremely severe course of hemolytic disease.

Amniocentesis.

Measuring the optical density of bilirubin in amniotic fluid obtained via amniocentesis is an invasive diagnostic technique that is no longer used due to its low informational value.

Treatment of Hemolytic Disease of the Fetus

Conservative Treatment

There are currently no effective conservative treatment methods aimed at reducing the severity of Rh isoimmunization and hemolytic disease of the fetus. Their use leads to a loss of time and the potential development of extremely severe forms of hemolytic disease of the fetus.

Intrauterine Intravascular Transfusion of Packed Red Blood Cells to the Fetus

The universally accepted and proven method for treating severe forms of hemolytic disease of the fetus is intrauterine intravascular transfusion of packed red blood cells to the fetus. In the history of perinatal medicine, this method is considered the most successful among all fetal treatment methods.

Cordocentesis and subsequent analysis of fetal blood represent a direct diagnostic method for assessing clinical and biochemical blood parameters. Analysis of fetal blood obtained via cordocentesis includes: determination of the fetal ABO blood group and Rh status, levels of hemoglobin and hematocrit, acid-base balance parameters, and the direct Coombs test.

Normal fetal hemoglobin values correspond to hemoglobin ≥ 0.84 MoM. Fetal anemia is classified as mild (Hb < 0.84 MoM), moderate (Hb < 0.65 MoM), and severe (Hb < 0.55 MoM). A decrease in hemoglobin by 15% or more indicates the development of severe anemia and is an indication for intrauterine transfusion of packed red blood cells to the fetus.

Packed red blood cells are prepared from group O, Rh(D)-negative donor blood and have a high hematocrit of 80—85%. The final volume of packed red blood cells to be transfused is calculated based on the fetoplacental blood volume for the given gestational age, the initial fetal Ht (or Hb) value, and the Ht (or Hb) value of the packed red cells. Intravascular transfusion of packed red blood cells to the fetus raises hematocrit and hemoglobin levels to normal values, which helps prevent the development or resolves existing fetal hydrops, thereby allowing the pregnancy to be prolonged to near-term.

In cases of pronounced edematous form of hemolytic disease of the fetus, the transfusion of packed red cells is supplemented by administering a 20% albumin solution to the fetus. Transfusion of

packed red blood cells to the fetus helps attenuate the maternal immune response by reducing the relative number of Rh-positive erythrocytes in the fetal circulation. To prevent excessive fetal movement, which is one of the main causes of complications during cordocentesis and subsequent intravascular transfusion, the neuromuscular blocking agent pipecuronium is administered to the fetus.

Potential complications during cordocentesis and intrauterine transfusion of packed red blood cells include: bleeding from the umbilical cord puncture site, umbilical vessel thrombosis, cord hematoma, placental abruption, acute fetal hypoxia, premature rupture of membranes, preterm birth, and infection. After completing the transfusion of the calculated volume of packed red cells, a fetal blood sample is taken to determine post-transfusion hematocrit and hemoglobin levels to assess the effectiveness of the intrauterine correction of anemia. The need for repeat transfusions to the fetus is determined by the gestational age at the time of the previous transfusion, the final fetal Ht value, and the dynamics of changes in the MCA-PSV.

Management of Pregnancies with HDF Requiring Intrauterine Transfusion

The treatment of pregnant women with HDF requiring intrauterine transfusion of packed red blood cells must be carried out in institutions equipped to care for preterm newborns (Level III institutions, “Perinatal Centers”).

Intrauterine transfusions can be performed multiple times. Typically, the last transfusion is administered at 33 weeks; however, in recent years, there has been a trend towards performing transfusions even at 35—36 weeks of gestation, allowing for delivery at 37 weeks. This approach reduces the number of complications associated with neonatal prematurity. Prior to performing an intrauterine transfusion or when planning delivery before 34 weeks of

gestation, a single course of prophylaxis for respiratory distress syndrome (RDS) is administered according to the following regimen: 4 intramuscular doses of dexamethasone (6 mg each) at 12-hour intervals or 3 intramuscular doses of dexamethasone (8 mg each) at 8-hour intervals. The total course dose of dexamethasone is 24 mg.

Indications for Hospitalization of Pregnant Women with HDF.

Severe fetal anemia (MCA-PSV ≥ 1.5 MoM) — for performing intrauterine transfusion of packed red blood cells up to 34 weeks of gestation (inpatient, emergency).

Moderate fetal anemia (MCA-PSV 1.3—1.49 MoM) — for monitoring and deciding on the need for intrauterine transfusion of packed red blood cells up to 34 weeks of gestation (inpatient, planned).

Need for monitoring and early delivery in a pregnant woman with Rh isoimmunization and moderate fetal anemia, for cervical ripening in preparation for labor at 36—37 weeks of gestation (inpatient, planned).

Management of Labor in Rh-Isoimmunized Pregnancies

The timing and method of delivery are determined individually in each case, taking into account obstetric history, fetal condition, and the capabilities of the obstetric and neonatal services at the specific maternity facility.

The following factors influence the course and outcome of HDF: gestational age at delivery (factor of prematurity and immaturity), presence of severe fetal heart failure, concurrent fetal infection, severity of anemia at birth, presence of fetal hydrops

In the presence of HDF, early delivery at 37—38 weeks is indicated, as delivery at an earlier gestational age due to functional organ immaturity (especially the liver) results in a more severe course of hemolytic disease. However, in cases of severe fetal anemia, the edematous form of HDF, or following intrauterine

transfusion of packed red cells, operative delivery at an earlier gestation is recommended, after a course of RDS prophylaxis. The choice of cesarean section is justified by the avoidance of additional fetal trauma and hypoxia during labor.

If the fetal condition is satisfactory, delivery via the natural birth canal is performed. Cesarean section is performed in the presence of additional obstetric complications (placenta previa, abnormal fetal position, etc.). It is advisable to conduct planned labor induction after cervical ripening (using mechanical dilators, mifepristone, or dinoprostone gel).

During labor, fetal condition is monitored (via CTG). Epidural analgesia is advisable. Given the tendency of the fetus and newborn with HDF to hemorrhage, the second stage of labor is managed gently. Immediately after birth, the infant is quickly separated from the mother to prevent massive transfer of Rh antibodies into the newborn's bloodstream. Blood is taken from the umbilical cord to determine bilirubin and hemoglobin levels, as well as the infant's blood group and Rh status. An indirect Coombs test is performed to detect the newborn's erythrocytes sensitized with antibodies (agglutination reaction of the infant's blood with specific Coombs serum). Subsequently, if exchange transfusion is necessary for newborns with hemolytic disease, the umbilical vessels are used; therefore, a clamp is not applied to the umbilical cord. The cord is ligated 2—3 cm from the umbilical ring.

Prevention of Rh Isoimmunization

A significant reduction in perinatal morbidity and mortality from hemolytic disease of the fetus is impossible without organizing measures for the timely and universal prevention of Rh isoimmunization during pregnancy and in the early postpartum period at the population level. It has been proven that the implementation of preventive methods in practice over 20 years in the United Kingdom has reduced the frequency of Rh isoimmunization almost 30-fold,

from 46 to 1.6 cases per 100,000 births. Furthermore, for the healthcare system, the costs associated with implementing the prevention of Rh isoimmunization are significantly lower compared to the costs of treating hemolytic disease of the fetus and newborn.

Non-Specific Prophylaxis

Preservation of the first and subsequent pregnancies in women with Rh-negative blood.

Prevention of transfusing any donor blood component preparations to female patients without considering the Rh compatibility of the donor's blood.

Specific Antenatal Prophylaxis of Rh Isoimmunization

Protocol for specific antenatal prophylaxis of Rh isoimmunization with anti-Rh(D) immunoglobulin during pregnancy.

After the first prenatal visit, determination of the woman's blood group and Rh status is indicated. If Rh-negative status is identified/confirmed, testing for the exclusion / detection of anti-Rh antibodies is indicated, as well as determination of the biological father's blood group and Rh status.

Prophylaxis of Rh isoimmunization is not indicated in cases where the father is Rh-negative (the pregnancy is managed as uncomplicated) and when the fetus has an Rh-negative genotype, confirmed by non-invasive testing of maternal blood.

In the absence of maternal Rh isoimmunization and with an Rh-positive or unknown paternal blood type, the next anti-Rh antibody test is indicated at 18 weeks of gestation.

If the mother shows no Rh isoimmunization at this gestational age, the next test is indicated at 28 weeks. In the absence of Rh isoimmunization at 28 weeks, antenatal prophylaxis is indicated — intramuscular administration of a single dose of anti-Rh(D) immunoglobulin (1250—1500 IU — 250—300 μg).

If prophylaxis was not administered at 28 weeks, it is indicated at the earliest possible time at any gestational age, provided anti-Rh antibodies are absent.

Following invasive diagnostic or therapeutic interventions during pregnancy (chorionic villus sampling, amniocentesis, cordocentesis, cerclage, fetal reduction in multifetal pregnancy, external cephalic version for breech presentation, conditions following abdominal trauma during pregnancy, obstetric hemorrhage) in the absence of maternal Rh isoimmunization, additional antenatal prophylaxis of Rh isoimmunization is indicated: administration in the first trimester — 625 IU (125 μg); in the second and third trimesters — 1250—1500 IU (250—300 μg) of anti-Rh(D) immunoglobulin.

Additional prophylaxis of Rh isoimmunization in early pregnancy should be performed directly after the completion of the aforementioned indications, and its administration does not preclude the scheduled administration of anti-Rh(D) immunoglobulin at 28 weeks.

If 12 weeks have passed since prophylaxis for the aforementioned indications (bleeding, cerclage, invasive interventions), repeat prophylaxis of Rh isoimmunization is recommended.

For up to 12 weeks after antenatal prophylactic administration of anti-Rh(D) immunoglobulin, trace levels of anti-Rh antibody titers may be detectable.

Administration of anti-Rh(D) immunoglobulin is recommended in cases of unsuccessful pregnancy termination: instrumental and medical abortion at the end of the first trimester; spontaneous and medical abortion in the second trimester; antenatal fetal death.

Protocol for Specific Antenatal Prophylaxis of Rh Isoimmunization with Anti-Rh(D) Immunoglobulin After Childbirth.

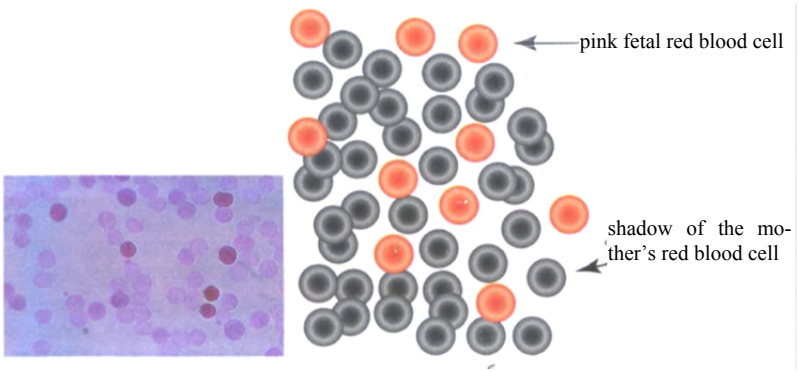
After childbirth, determination of the newborn's blood group and Rh status is indicated. If the newborn is Rh-negative, specific prophylaxis for Rh isoimmunization is not required.

If the newborn is Rh-positive, specific prophylaxis for Rh isoimmunization is indicated by intramuscular administration of anti-Rh(D) immunoglobulin at a dose of 1500 IU (300 μg). It is optimal to administer this immediately after receiving the results of the infant's blood test, and preferably no later than 72 hours after delivery (ideally within the first two hours).

If prophylaxis was not administered for any reason, administration of anti-Rh(D) immunoglobulin is possible up to 10 days postpartum.

Method for Selecting the Dose of Anti-Rh(D) Immunoglobulin.

To calculate the dose of anti-Rh immunoglobulin, it is necessary to consider the volume of fetal blood that has entered the maternal circulation through the placenta. A method for estimating this dose is the Kleihauer-Betke test (Fig. 7). This test is based on the phenomenon of eluting maternal hemoglobin (HbA) from erythrocytes in a citrate-phosphate buffer, while fetal hemoglobin (HbF) is not eluted.



a *b*

Fig. 7. Kleihauer-Betke Test: Microscopic Slide (*a*) and Schematic Diagram (*b*)

After appropriate processing of the maternal peripheral blood smear, the number of fetal cells is counted. If the volume of feto-maternal hemorrhage does not exceed 25 ml, a standard dose of 1500 IU (300 mcg) of anti-Rh0(D) immunoglobulin is administered; for a volume of 25—50 ml, the dose is doubled to 3000 IU (600 mcg).

In the absence of the technical capability to determine the volume of fetal blood that has entered the maternal circulation via the Kleihauer-Betke test, it is recommended to double the dose of anti-Rh0(D) immunoglobulin in the following cases: placental abruption, manual examination of the postpartum uterus, and cesarean section accompanied by significant blood loss.

Rh-isoimmunized women with a history of fetal / newborn loss due to hemolytic disease should be informed about the possibility of selecting and performing the selective transfer of embryos with an Rh-negative genotype in IVF cycles utilizing preimplantation genetic testing.

Self-assessment test questions

Choose the single correct answer.

1. An erroneous factor for prior sensitization in rhesus conflict is:
1) abortion
2) miscarriage
3) childbirth
4) immunoprophylaxis
5) blood transfusions

2. The method used for antenatal diagnosis of hemolytic disease of the fetus (HDF) is:
1) cardiotocography
2) determination of the fetal biophysical profile
3) amniocentesis and measurement of bilirubin optical density
4) doppler ultrasonography and measurement of peak systolic velocity in the middle cerebral artery (MCA-PSV)
5) auscultation of fetal heart rate

3. Administration of anti-rhesus immunoglobulin during pregnancy and after delivery is indicated in the case of:

1) mother Rh(–), child Rh(+), no antibodies present
2) mother Rh(–), child Rh(+), antibodies present
3) mother Rh(–), child Rh(–), no antibodies present
4) mother Rh(+), child Rh(–), no antibodies present
5) mother Rh(+), child Rh(+), no antibodies present

4. Administration of anti-rhesus immunoglobulin is indicated for all pregnant women with Rh-negative blood and a fetus of unknown Rh status, in the absence of antibody titer, at a gestational age of:

1) 12 weeks
2) 18 weeks
3) 20 weeks
4) 24 weeks
5) 28 weeks

5. The D antigen appears at ___ weeks of gestation.

1) 4—6 weeks
2) 6—8 weeks
3) 8—10 weeks
4) 10—12 weeks
5) 12—14 weeks

6. The frequency of fetal erythrocyte passage through the placental barrier into the maternal circulation during the first trimester of pregnancy is:

1) 5—7%
2) 15—20%
3) 25%
4) 45%
5) 90%

7. The development of hemolytic disease of the newborn (HDN) is possible with the following combination of maternal and child blood group and rhesus status:

1) mother A (II) Rh(–), child O (I) Rh(+)
2) mother B (III) Rh(+), child A (II) Rh(–)
3) mother O (I) Rh(+), child O (I) Rh(–)
4) mother A (II) Rh(+), child O (I) Rh(–)
5) mother B (III) Rh(+), child O (I) Rh(+)

8. Antibody formation and the development of HDN are most often caused by the following antigen of the rhesus system:

1) C
2) D
3) E
4) c
5) e

9. The reason why rhesus conflict rarely develops during a first pregnancy is:

1) fetal erythrocytes do not cross the placenta
2) Rh antibodies do not cross the placenta
3) Rh antibodies do not have time to form during the first pregnancy
4) due to the state of physiological immune suppression in the pregnant woman, the primary immune response is reduced
5) as a result of mandatory prophylaxis with anti-Rhesus immunoglobulin for all pregnant women with Rh-negative blood

10. The dose of anti-rhesus immunoglobulin for specific prophylaxis in pregnant women with Rh-negative blood and a fetus of unknown Rh status, in the absence of antibody titer in the third trimester, is:

1) 625 IU
2) 725 IU
3) 1250—1500 IU
4) 1500—1750 IU
5) 1750—2000 IU

Standard answers to test tasks for self-assessment

Task 1	4
Task 2	4
Task 3	1
Task 4	5
Task 5	4
Task 6	1
Task 7	1
Task 8	2
Task 9	3
Task 10	3

Part II

HEMOLYTIC DISEASE OF THE NEWBORN. DIAGNOSIS. TREATMENT

Definition and Key Links in the Pathogenesis of HDN

Hemolytic Disease of the Fetus and Newborn (HDF/HDN) is an isoimmune hemolytic anemia that occurs in cases of incompatibility between maternal and fetal blood based on erythrocyte antigens (Ag), where the antigens are located on fetal erythrocytes and antibodies (Ab) to them are produced in the maternal organism.

In Russia during 2014—2016, HDN was diagnosed in 0.9—1 % of newborns [5].

An immunological conflict is possible if antigens present on fetal erythrocytes are absent on the mother's cell membranes. Thus, the immunological prerequisite for the development of HDN is the presence of an Rh-positive fetus in an Rh-negative pregnant woman. In an immunological conflict due to blood group incompatibility, the mother most often has blood group O(I), and the fetus has group A(II) or (less commonly) B(III). More rarely, HDN develops due to mismatches between the fetus and the pregnant woman in other blood group systems (Duffy, Kell, Kidd, Lewis, MNSs, etc.).

Previous isosensitization due to abortions, miscarriages, ectopic pregnancies, or deliveries predisposes to the entry of fetal erythrocytes into the maternal bloodstream and the occurrence of an immunological conflict in cases of antigenic blood factor incompatibility. During these events, the mother's immune system produces antibodies against erythrocyte antigens. If the antibodies belong to immunoglobulin class G (subclasses IgG1, IgG3, IgG4) — they cross the placenta unimpeded. As their concentration in the

blood increases, so does the likelihood of developing hemolytic disease of the fetus and newborn. Antibodies of the IgG2 subclass have limited transplacental transport ability, while antibodies of the IgM class, which include α- and β-agglutinins, do not cross the placenta.

The manifestation of HDN due to the Rh factor typically occurs in subsequent pregnancies, while the development of HDN resulting from a conflict based on blood group factors is possible even in a first pregnancy.

In the development of HDN due to the ABO system, hemolysis of erythrocytes occurs due to the entry of maternal anti-A antibodies into the blood of a group A(II) child; less commonly, it happens with the entry of anti-B antibodies into the blood of a group B(III) child. However, the penetration of anti-B antibodies leads to a more severe course of the disease, often requiring exchange transfusion.

The severity of the infant's condition and the risk of kernicterus in ABO-system HDN are less pronounced compared to Rh-factor HDN. This is explained by the fact that group antigens A and B are expressed by many cells in the body, not just erythrocytes, which leads to the binding of a significant amount of antibodies in non-hematopoietic tissues and hinders their hemolytic effect.

Classification of Hemolytic Disease of the Newborn

Hemolytic Disease of the Fetus and Newborn (HDFN) is an isoimmune hemolytic anemia that occurs in cases of incompatibility between maternal and fetal blood for red cell antigens (Ags). In this condition, the antigens are located on fetal erythrocytes, while antibodies (Abs) to them are produced in the maternal body.

In Russia, HDFN was diagnosed in 0.9—1 % of newborns in 2014—2016 [5].

An immunological conflict can arise if antigens present on fetal erythrocytes are absent on the mother's cell membranes. Thus, the

immunological prerequisite for the development of HDFN is an Rh-positive fetus in an Rh-negative pregnant woman. In cases of immunological conflict due to ABO incompatibility, the mother most often has blood group O(I), and the fetus has group A(II) or (less frequently) B(III). More rarely, HDFN develops due to incompatibility between the fetus and the pregnant woman for other blood group systems (Duffy, Kell, Kidd, Lewis, MNSs, etc.).

The entry of fetal erythrocytes into the maternal bloodstream and the onset of an immunological conflict in cases of blood factor antigenic incompatibility are predisposed by prior isoimmunization due to abortions, miscarriages, ectopic pregnancies, or deliveries, during which the maternal immune system produces antibodies against erythrocyte antigens. If the antibodies belong to immunoglobulin class G (subclasses IgG1, IgG3, IgG4), they freely cross the placenta. As their concentration in the blood increases, the likelihood of developing hemolytic disease of the fetus and newborn rises. Antibodies of the IgG2 subclass have a limited capacity for transplacental transport, while antibodies of the IgM class, which include α- and β-agglutinins, do not cross the placenta.

The manifestation of Rh factor-related HDFN typically occurs during repeated pregnancies, whereas the development of HDFN resulting from blood group factor conflict is possible even during the first pregnancy.

In ABO system-related HDFN, hemolysis of erythrocytes occurs due to the entry of maternal anti-A antibodies into the bloodstream of a group A(II) child; less frequently, it happens when anti-B antibodies enter the bloodstream of a group B(III) child. However, the penetration of anti-B antibodies leads to a more severe disease course, often requiring exchange transfusion.

The severity of the infant's condition and the risk of kernicterus in ABO-related HDFN are less pronounced compared to Rh factor-related HDFN. This is explained by the fact that A and B blood group antigens are expressed on many cells throughout the body, not just erythrocytes. This leads to the binding of a significant number of antibodies in non-hematopoietic tissues and prevents their hemolytic effect.

Several different classifications of hemolytic disease of the fetus and newborn have been published in domestic and foreign literature. The most well-known are the classifications by Academician L. S. Persianinov (1981) and Academician G. M. Savelyeva (2013).

For formulating the final clinical diagnosis of HDFN in a newborn, the following classification is recommended/

1. **Based on the type of immunological incompatibility** of maternal and fetal erythrocytes causing HDFN:
   - Rh factor incompatibility;
   - ABO system incompatibility (blood group incompatibility);
   - incompatibility for rare blood factors.
2. **According to the leading clinical manifestations:**
   - **anemic form** (hemolytic anemia without jaundice and hydrops);
   - **icteric form** (hemolytic anemia with jaundice);
   - **edematous form** (hemolytic anemia with hydrops).

The anemic form of HDFN (which is not characterized by pathological jaundice and hydrops) most often corresponds to a mild degree of severity. The icteric form of HDFN can vary in the degree of jaundice severity — from mild to severe. The edematous form of HDFN represents the most severe form.

3. **Based on the presence or absence of complications:**
   - **complicated form** (possible complications: kernicterus, inspissated bile syndrome, hemorrhagic syndrome, and other conditions requiring additional pathogenetic treatment);
   - **uncomplicated form.**

Clinical Presentation.

1. **Anemic form of HDFN** — the least common and mildest form of the disease:
   - pallor of the skin;
   - lethargy, poor sucking;
   - tachycardia;
   - muffled heart sounds;

- systolic murmur;
- enlargement of the liver and spleen is typically absent at birth; hepatolienal syndrome is detected during follow-up; its severity is moderate.

2. **Icteric form of HDFN** — the most frequently detected form:

- the general condition of the child depends on the severity of hemolysis and the degree of hyperbilirubinemia;
- very rarely, yellowish-stained amniotic fluid, umbilical cord membranes, and vernix caseosa are noted at birth;
- during the initial examination, some infants show a moderately pronounced hepatolienal syndrome, while in others, an increase in palpable liver and spleen size is detected 6—12 hours after birth;
- in all cases, early onset of jaundice is characteristic (from birth to within 24 hours of life);
- pallor of the skin and visible mucous membranes at birth is moderate but can rapidly progress after birth in a number of cases;
- the degree of liver and spleen enlargement detected during follow-up generally correlates with the degree of decrease in hemoglobin concentration and the hourly rate of bilirubin increase during the first day of life. The classification of the icteric form of HDFN by severity is presented in (Table 1).

Table 1

Classification of Severity of the Icteric Form of HDN

Severity	Onset of jaundice	Cord blood bilirubin	Hourly bilirubin increment
Mild	Days 1—2	< 51 μmol/L	Up to 4—5 μmol/L
Moderate	First hours	> 68 μmol/L	6—10 μmol/L
Severe	In utero	> 68 μmol/L	Data not specified / Requires intensive monitoring

3. **Edematous form of HDFN** — the most severe form, characterized from the first minutes of life by the following signs:

- at delivery, yellowish-stained amniotic fluid, umbilical cord membranes, and vernix caseosa are often detected;
- presence of widespread edema, including anasarca, ascites, and hydropericardium (corresponding to grade II—III edematous syndrome);
- marked pallor of the skin and visible mucous membranes; in some cases — mild jaundice;
- hepatomegaly and splenomegaly.

Most infants have low Apgar scores; due to the presence of severe respiratory and cardiovascular failure, the majority require a complex of primary resuscitation measures in the delivery room. This form is often complicated by the development of DIC (disseminated intravascular coagulation) syndrome and shock.

Without early initiation of adequate intensive therapy, including early partial exchange transfusion, the progression of multiorgan failure quickly leads to neonatal death.

In recent years, due to the widespread introduction into obstetric practice of intrauterine blood transfusions for early detection of hemolytic disease of the fetus, the incidence of the edematous form of HDFN has somewhat decreased.

Complications of HDFN

Bilirubin Encephalopathy (BE) — damage to the nervous system resulting from unconjugated bilirubin injuring the neurons of the brain nuclei. It typically develops between the 4^{th} and 7^{th}—10^{th} days of life. Historically, 4 phases of BE are distinguished.

Phase 1 — Bilirubin Intoxication. Appears within the first hours of the disease, lasting 1—2 days. Intoxication, lethargy, regurgitation, vomiting, and apneic episodes increase. Reduced muscle tone and appetite, refusal to feed, paucity of movements and emotional coloring of the cry (monotonous cry), incomplete Moro

reflex (only the first phase), pathological yawning, "wandering gaze," automatic chewing movements of the lips. With immediate provision of exchange transfusion, the changes that have arisen are reversible.

Phase 2 — Spastic. Onset — day 3—4 of life / disease. Appearance of classical signs of kernicterus in the newborn — 'fencing' or 'decerebrate' posture (forced body position with opisthotonus, "stiff" limbs, and clenched fists), head retraction, neck stiffness, muscle hypertonia, hyperesthesia, convulsions, periodic agitation and a sharp "cerebral" cry, bulging of the anterior fontanelle; the Moro reflex and visible reaction to loud sound, sucking reflex disappear; apnea, bradycardia, oculomotor symptoms (nystagmus, setting sun sign). This phase lasts from several days to several weeks. CNS damage is irreversible.

Phase 3 — Apparent Well-being. Lasts from several days to several weeks. Develops at 2^{nd}—3^{rd} months of life. The intensity of spasticity decreases, jaundice sharply diminishes.

Phase 4. The period of forming the clinical picture of neurological complications (usually the 3^{rd}—5^{th} month of life). Characterized by severe organic changes of the CNS, spastic pareses and paralyses, severe oculomotor symptoms, hearing loss, developmental delay, enamel hypoplasia of the primary teeth.

Cholestasis Syndrome. Can develop at any time, more often after an exchange transfusion (ET). Jaundice acquires a greenish tint, the liver increases in size compared to the previous examination, and the color of urine intensifies. This is accompanied by an increase in the direct bilirubin fraction (more than 20 % of the total level) and other biochemical markers of cholestasis: enzymes GGT, ALP, cholesterol. Cytolysis syndrome (elevated ALT, AST) may also develop.

Diagnosis

The diagnosis of Rh factor-related HDFN can be established in the first hours of a child's life based on history (increase in anti-D antibody titer in Rh(–) women during pregnancy, ultrasound signs

of fetal hemolytic disease). All women with a negative Rh factor are indicated for dynamic monitoring of immune antibody levels in the blood during pregnancy. One of the important non-invasive methods for assessing the condition of the fetus in mothers with Rh sensitization is Doppler measurement of blood flow in the fetal middle cerebral artery, as there is a direct correlation between the peak systolic velocity in the middle cerebral artery and the severity of hemolytic disease, primarily related to the fetal hematocrit level.

Hemolytic disease due to the ABO system (ABO-HDFN), as a rule, does not have specific signs in the first hours after birth.

If the mother's blood is characterized by a negative Rh factor or belongs to group O(I), the newborn must undergo testing for total bilirubin concentration in cord blood and determination of blood group and Rh factor. If HDFN is suspected, the following laboratory tests should be performed.

Blood group and Rh affiliation of the mother and child.

Complete Blood Count (CBC). Characteristic findings for HDFN include:

1) anemia;

2) increased nucleated red blood cells (reflects active erythropoiesis; during automated CBC analysis, these are often identified by hematology analyzers as lymphocytes, which reduces the diagnostic value of this indicator);

3) reticulocytosis (can reach up to 40 %). A low reticulocyte count may be observed after intrauterine blood transfusion and in Kell system alloimmunization (however, as with the assessment of erythroblast count, when using hematology analyzers, verification by visual examination of a peripheral blood smear by a laboratory physician is required);

4) polychromasia;

5) anisocytosis;

6) spherocytosis (more often detected in ABO-related HDFN) and cell fragmentation;

7) there may be leukocytosis, increased immature leukocyte forms, leukopenia, and thrombocytopenia.

Biochemical Blood Analysis (total bilirubin and fractions, albumin, glucose level; other parameters (bilirubin fractions, acid-base status, electrolytes, etc.) — as indicated);

- HDFN is characterized by an increased level of total bilirubin due to the indirect fraction;
- an albumin level < 30 g/L is a factor that increases the risk of bilirubin toxicity;
- an increase in the direct bilirubin fraction, ALT, AST, GGT, and alkaline phosphatase is not characteristic (except in cholestasis syndrome).

Serological Tests.

Coombs Test (Antiglobulin Test).

The essence of this method is that antiglobulin serum, containing antibodies against human immunoglobulins, causes agglutination when reacting with erythrocytes sensitized by incomplete antibodies. Depending on whether the antibodies are fixed on the surface of erythrocytes or are in free state in the plasma, a direct or indirect Coombs test is used.

For the diagnosis of HDFN, the **direct Coombs test** is usually used. The direct Coombs test becomes positive in the presence of fixed antibodies on the erythrocyte surface, which is typically observed in Rh-related HDFN. Due to the small number of antibodies fixed on erythrocytes in ABO-related HDFN, a weakly positive direct Coombs test is more often observed on day 1 of life, which may become negative 2—3 days after birth.

The **indirect Coombs test** is designed to detect incomplete antibodies present in the tested serum. This is a more sensitive test for detecting maternal iso-antibodies than the direct Coombs test. The indirect Coombs test may be used in individual cases where the cause of hemolysis is unclear.

It should be remembered that the **severity of the Coombs test reaction does not correlate with the severity of jaundice!**

Differential diagnosis is carried out with the following conditions:

- hereditary hemolytic anemias due to erythrocyte morphology disorders (spherocytosis, elliptocytosis, stomatocytosis), erythro-

cyte enzyme deficiencies (glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione peroxidase, pyruvate kinase), hemoglobin synthesis anomalies (alpha-thalassemia);

- post-hemorrhagic anemias;
- non-immune hydrops fetalis;
- infections: cytomegalovirus, parvovirus B19, syphilis, toxoplasmosis;
- metabolic disorders: deficiency of galactose-1-phosphate uridylyltransferase (galactosemia), hypothyroidism, tyrosinemia.

Treatment

General Principles and Treatment Algorithm for HDFN in the First 24 Hours.

- The basis for transferring a child with HDFN to a neonatal intensive care unit (NICU) is the presence of indications for an exchange blood transfusion (EBT) at any age in the neonatal period.
- There are two types of treatment for HDFN: conservative and operative. Conservative treatment includes intravenous immunoglobulin (IVIG) administration and phototherapy; operative treatment is EBT.
- For HDFN, early, intensive (high-dose) continuous phototherapy is indicated.
- To reduce the intensity of hemolysis and the need for EBT in the first hours of life, administration of standard human immunoglobulin may be performed.
- **EBT is indicated:** in the **edematous form** of HDFN — for timely correction of severe anemia and prevention of progressive hyperbilirubinemia; in the **icteric form** — when phototherapy is ineffective, to prevent the development of bilirubin encephalopathy.
- Preparation for EBT in the edematous form of HDFN should be carried out even before the birth of the child; the procedure should be started within the first 20 minutes after birth.

• One of the main laboratory criteria for choosing conservative or operative tactics in children with the icteric form are: the initial hemoglobin level in cord blood, the initial concentration and hourly increment of total bilirubin (TB). In this case, direct (conjugated) bilirubin should **not** be subtracted from the total bilirubin level.

• To determine the management tactics for newborns with HDFN between 24 and 72 hours of life, the 2013 National Guide to Neonatology recommends using the table by Maisels MJ [10] (Table 2).

• If clinical signs characteristic of **phase 1 or 2 of bilirubin encephalopathy** appear in a newborn with hyperbilirubinemia (after excluding other causes of neurological disorders), EBT is indicated even if the total serum bilirubin level is below the values presented in the table.

• Management of newborns with HDFN who underwent **intrauterine blood transfusion** is carried out according to the general rules and principles of HDFN treatment.

• **Infusion Therapy.** Toxic effects are attributed to **indirect (fat-soluble) bilirubin**; therefore, its level cannot be reduced by administering glucose solutions. There is no convincing evidence that increasing fluid intake affects serum bilirubin concentration. The exception is children with signs of dehydration or hypoglycemia.

Table 2

Indications for Phototherapy and EBT in Newborns Diagnosed with HDFN Aged 24—168 Hours, Depending on Birth Weight (Total Serum Bilirubin Level)

Birth Weight (grams)	Phototherapy	EBT
< 1500	85 μmol/L	220 μmol/L
1500—1999	140 μmol/L	275 μmol/L
2000—2500	190 μmol/L	300 μmol/L
> 2500	235 μmol/L	340 μmol/L

Treatment (Continued). Management Tactics for the Edematous Form of HDFN.

- In the edematous form of HDFN, the umbilical cord is clamped immediately (within 5—10 seconds) to prevent the development of hypervolemia.
- Children with the edematous form of HDFN at birth have severe anemia, respiratory, and hemodynamic disturbances. Stabilizing these patients requires a high level of intensive management and coordination by the neonatal team. The risk of bilirubin toxicity immediately after birth is not their primary problem.
- Immediate intubation and mechanical ventilation with positive inspiratory pressure are indicated. In preterm infants, administration of surfactant drugs may be considered.
- Ineffective mechanical ventilation against a background of severe anasarca is an indication for thoraco- and paracentesis to drain the cavities and improve respiratory function. Excessive removal of ascitic fluid is avoided due to the risk of systemic hypotension. To prevent hemorrhagic complications, hemostatic therapy is recommended (ethamsylate; in life-threatening situations, vitamin K). During paracentesis, it is considered that the liver and spleen are enlarged.
- Given the severe anemia and hemic hypoxia of the child's tissues and organs, immediately after primary stabilization, a **partial exchange transfusion** must be carefully performed, avoiding circulatory overload in the setting of heart failure (hypoxic myocardial damage).
- The partial exchange transfusion is recommended to be started as soon as possible after birth — no later than 20 minutes of life. In the most severe patients, the procedure is performed in the delivery room.
- Partial exchange transfusion is performed by replacing 45—90 ml/kg of the child's blood with an equivalent volume of **O(I) Rh-negative packed red blood cells**. The child's initial hemoglobin level may not be considered for this decision. The technique is

similar to the full exchange transfusion described below, except that the removed blood volume is replaced exclusively with packed red blood cells.

- Subsequently, syndrome-targeted therapy is continued, aimed at stabilizing respiratory function, central hemodynamic parameters, correcting major metabolic disturbances (hypoglycemia, hypocalcemia, hyponatremia, hyperkalemia, hypoproteinemia, acidosis), improving kidney function, and preventing/treating hemorrhagic complications. This syndrome-targeted therapy is **mandatorily** carried out against the background of **continuous, intensive (high-dose) phototherapy**.
- After stabilization of the child's general condition — which may take from 1 to 12 hours — the procedure should be continued to exchange the child's blood for donor blood in a volume equivalent to **2 blood volumes (2 BV)**.
- If severe anemia develops after the exchange transfusion (hemoglobin level below 120 g/L), but the hourly bilirubin increment and absolute level of hyperbilirubinemia do not reach critical values, a **simple blood transfusion (transfusion of packed red blood cells)** is indicated.

Conservative Therapy.

1. **Phototherapy (PT)** — the most effective method of conservative therapy for HDFN. Features of PT in HDFN:

- standard lamps, fiberoptic, and LED phototherapy can be used; it is advisable to combine several methods;
- the light source is placed 50 cm above the child. To enhance the effect, the lamp can be moved closer to a distance of 10—20 cm from the child under constant medical supervision and body temperature monitoring;
- phototherapy for HDFN (especially in children at risk for EBT) should be performed **continuously**;
- the child's body surface should be maximally exposed during PT. A diaper may be left in place;
- eyes and genitals must be protected with light-blocking material;

o the daily fluid volume received enterally or parenterally should be increased by **10—20 %** compared to the child's physiological requirement;

o 12 hours after stopping phototherapy, a control bilirubin test must be performed;

o phototherapy is conducted **before, during** (using a fiberoptic system), and **after** the exchange blood transfusion.

Intravenous Immunoglobulins (IVIG).

High doses of standard immunoglobulins block the Fc-receptors of the reticuloendothelial system cells, thereby reducing hemolysis and consequently the bilirubin level, which in turn decreases the number of EBTs required. Human immunoglobulin preparations are administered to newborns with HDFN according to the following scheme:

- in the first hours of life, administered intravenously slowly (preferably over 2 hours), strictly following the drug's instructions;
- dose: **0.5—1.0 g/kg** (average 0.8 g/kg)*;
- if necessary, repeat administration of immunoglobulin is performed 12 hours after the previous dose;
- immunoglobulin administration for HDFN is possible within the **first 3 days of life**.

2. **Supplemental Feeding.** Not recommended for breastfed infants. It is only possible if breast milk is insufficient to increase the daily volume by 10—20 %. If the child's condition does not allow increasing fluid volume enterally, only then is infusion therapy initiated.

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* If a dose exceeding the one specified in the drug's instructions is prescribed, this action must be thoroughly justified in the medical record, and a collegial approval for "off-label" therapy must be obtained. The use of "off-label" therapy also requires mandatory documentation of **voluntary informed consent** from the patient's legal representative, detailing the specifics of such therapy, potential risks and side effects, and explaining the right to refuse "off-label" treatment.

3. **Choleretic Therapy** is indicated in case of cholestasis syndrome developing against the background of HDFN. Conducted only with **ursodeoxycholic acid** in suspension form at a dose of 20—30 mg/kg/day.

Operative Treatment: Exchange Blood Transfusion (EBT)

The exchange blood transfusion (EBT) is primarily aimed at removing free (indirect) bilirubin. The goal of the procedure is to prevent the development of kernicterus when conservative therapy is ineffective. The most effective removal of bilirubin from the blood is achieved by replacing the patient's blood with donor blood components (packed red blood cells and plasma) in a volume of **2 blood volumes (2 BV)**.

If there are indications for EBT in children with the icteric form of HDFN, the procedure is always performed in the **standard volume (replacement of 2 BV)**.

Preparation and Conduct of the Procedure.

1. Obtaining informed parental consent for the EBT is mandatory.

2. The procedure is performed in a Neonatal Intensive Care Unit (NICU) (in level I perinatal care obstetric facilities — in a procedure room).

3. Before starting the procedure, in critically ill newborns, standard intensive therapy methods must correct acidosis, hypoxemia, hypoglycemia, electrolyte imbalances, hemodynamic disturbances, hypothermia, etc.

4. The EBT is performed by a team of at least two people: a physician (neonatologist / anesthesiologist-intensivist / pediatriccian) and a pediatric nurse.

5. A procedural note for the EBT must be documented in the medical record.

6. Children should not receive enteral nutrition for the last 3 hours before the intended start of the procedure.

7. A cleansing enema must be performed before the procedure.

8. Immediately before the procedure, a nasogastric tube must be inserted to periodically aspirate gastric contents.

9. Placement of a urinary catheter is advisable to assess urine output rate and color.

10. The procedure is performed under strict aseptic and antiseptic conditions.

11. Constant monitoring of vital functions (HR, RR, SpO2, BP, body temperature) is necessary throughout the preparation, procedure, and subsequent management.

12. Donor blood and/or its components for EBT are transfused at a rate of 160—180 ml/kg of body weight for a term infant and 170—180 ml/kg for a preterm infant (an example calculation is provided in Appendix 1).

13. The ratio of packed red blood cells / suspension to fresh frozen plasma is 2 : 1.

14. According to RF Government Resolution No. 797 dated 22.06.2019 "On Approval of the Rules for the Clinical Use of Donor Blood and/or Its Components," when transfusing donor blood and/or erythrocyte-containing components to newborns:

o leukocyte-depleted erythrocyte-containing components are transfused (erythrocyte suspension, packed red blood cells, washed erythrocytes, thawed and washed erythrocytes);

o when selecting donor blood components for transfusion, it is considered that the mother is an undesirable donor of fresh frozen plasma for the newborn, as her plasma may contain alloantibodies against the newborn's erythrocytes, and the father is an undesirable donor of erythrocyte-containing components, as antibodies against the father's antigens may be present in the newborn's blood, having crossed the placenta from the maternal bloodstream;

o transfusion of cytomegalovirus-negative erythrocyte-containing components is most preferable;

o transfusion of pathogen-inactivated fresh frozen plasma is **not permitted**;

o for exchange transfusion, erythrocyte-containing components with a storage time of **no more than 5 days** from the date of preparation are used;

o selection of donor blood components based on alloantibody specificity is as follows:

▪ for HDFN caused by anti-D alloimmunization, use single-group Rh-negative erythrocyte-containing components and single-group Rh-negative fresh frozen plasma;

▪ for ABO incompatibility, transfuse washed erythrocytes or erythrocyte suspension and fresh frozen plasma (Appendix 2), matching the child's Rh status and phenotype;

▪ for simultaneous ABO and Rh incompatibility, transfuse washed erythrocytes or O(I) Rh-negative erythrocyte suspension and AB(IV) Rh-negative fresh frozen plasma;

▪ for HDFN caused by alloimmunization to other rare erythrocyte antigens, individual donor blood selection is performed.

Order of Conducting EBT:

• place the child under a radiant heat source;

• secure the child's limbs with firm swaddling, leaving the abdominal skin exposed;

• insert an umbilical catheter with a pre-attached three-way stopcock under strict aseptic technique and secure it;

• if umbilical vein catheterization is contraindicated, perform EBT through any other central vein;

• donor blood components must be pre-warmed to 36—37 °C;

• the first portion of withdrawn blood must be taken for biochemical analysis of bilirubin level;

• subsequently, gradual withdrawal of the child's blood and replacement of the withdrawn volume are performed sequentially;

• the volume of a single withdrawal and a single replacement should not exceed **5 ml/kg**, under mandatory monitoring of hemodynamics, respiration, and kidney function;

• the speed of a single withdrawal is 3—4 ml/min;

• for every 2 syringes of packed red cells, administer 1 syringe of fresh frozen plasma;

• after every 100 ml of replacement fluid (packed red cells and plasma), administer 1.0—2.0 ml of 10% calcium gluconate or 0.5 ml of 10% calcium chloride, pre-diluted in 5.0 ml of 5% glucose (**only between syringes of packed red cells!**);

• before ending the procedure, collect blood for bilirubin testing;

• the procedure duration is at least 2 hours;

• as a result of the procedure (accounting for blood taken for analysis), the total volume of administered donor blood components must equal the total volume of the child's withdrawn blood.

NB! A **more than 2-fold reduction** in bilirubin by the end of the procedure indicates unequivocal effectiveness of the EBT.

Post-Procedure Period:

• continue monitoring vital functions;

• initiate enteral feeding no earlier than 3—4 hours after EBT;

• continue phototherapy;

• continue supportive therapy;

• removing the umbilical catheter immediately after the procedure is not recommended due to the probability of needing a repeat EBT;

• monitor bilirubin level 12 hours after EBT, then as indicated, but at least once every 24 hours until day 7 of life;

• monitor blood glucose 1 hour after EBT, then as indicated;

• according to Order of the Ministry of Health of the RF No. 183n, after transfusion, the donor container with the remaining donor blood / components (5 ml) and the tube with the recipient's blood used for compatibility testing must be stored for 48 hours at 2—6 °C in refrigeration equipment.

Complications that may arise during EBT.

1. **Cardiovascular:** arrhythmia, volume overload, congestive heart failure, circulatory arrest.

2. **Hematological:** heparin overdose, neutropenia, thrombocytopenia, graft-versus-host reaction.

3. **Infectious:** bacterial and viral infections.

4. **Metabolic:** acidosis, hypocalcemia, hypoglycemia, hyperkalemia, hypernatremia.

5. **Vascular:** embolism, thrombosis, necrotizing enterocolitis, portal hypertension, perforation of umbilical vessels.

6. **Systemic:** hypothermia.

Ineffective and Potentially Harmful Treatment Methods for HDFN that Should Be Excluded from Clinical Practice:

- **administration of albumin solution.** There is no evidence that albumin infusion improves long-term outcomes in infants with severe hyperbilirubinemia; therefore, its routine use is not recommended;
- **phenobarbital** — its effect in HDFN is unproven; its use is not permissible;
- **other medications** ("Essentiale," "Liv-52," and other "hepatoprotectors") — their use in HDFN is unproven and not permissible.

Discharge Criteria for Infants with HDFN.

1. Discharge from the maternity hospital to home or transfer to a secondary (level II) care facility is carried out in accordance with the Order of the Ministry of Health of Russia dated November 1, 2012, No. 572n "On Approval of the Procedure for Providing Medical Care in the Field of "Obstetrics and Gynecology" and the Order of the Ministry of Health of Russia dated November 15, 2012, No. 921n "On Approval of the Procedure for Providing Medical Care in the Field of "Neonatology".

2. Discharge home is only possible for **term newborns with mild HDFN**, after achieving a sustained decrease in blood bilirubin concentration 12 hours after stopping phototherapy and in the absence of clinical and laboratory signs of anemia.

3. In all cases of HDFN **other than those specified in point 2**, transfer of the child to a secondary (level II) care facility is indicated.

4. **Criteria for discharging a child with HDFN from the secondary care facility to home:**

- satisfactory condition of the child;
- no indications for the treatment of hyperbilirubinemia;

o it is unlikely that indications for blood transfusion will arise within the next month after discharge from the hospital.

5. After discharge from the hospital, during the first 3—4 months of life, it is recommended to monitor the hemoglobin level in a child with HDFN, on average once every 2 weeks (more frequently if indicated).

Note: During the neonatal period, vaccination of children with HDFN against hepatitis B and tuberculosis is contraindicated.

Prognosis

The most unfavorable prognosis is observed in the **edematous form of HDFN**, with mortality reaching up to **30 %**.

Over 90 % of children with HDFN who received timely treatment, including intrauterine blood transfusion, do not have subsequent neurological deviations. The development of **bilirubin encephalopathy** determines an unfavorable neurological outcome.

In patients who underwent intrauterine blood transfusion for HDFN, the frequency of hearing impairment is registered **5—10 times higher** than in the general population.

In children with HDFN, **severe anemia** requiring red blood cell transfusion may develop during the first month of life. One mechanism for the development of this anemia is the suppression of erythropoiesis due to the transfusion of red blood cells containing adult hemoglobin, which leads to a decrease in erythropoietin levels.

Self-assessment test questions

1. A characteristic feature of bilirubin metabolism in newborns is:
1) destroyed erythrocytes are the only source of bilirubin
2) erythrocyte hemolysis is reduced
3) bilirubin formation is reduced
4) increased level of ligandin
5) increased activity of the enterohepatic circulation

2. A characteristic feature of HDN due to abo incompatibility is:

1) rarely develops during the first pregnancy
2) the edematous form of the disease does not occur
3) often combined with Rh conflict
4) postnatal forms are often complicated by bilirubin encephalopathy
5) does not require exchange transfusion

3. A symptom complex characteristic of the bilirubin intoxication phase is:

1) muscle hypotonia, hyporeflexia
2) tremor, convulsions
3) development of paresis, paralysis
4) neck rigidity, opisthotonus
5) cerebral cry, sunset sign, nystagmus

4. A symptom complex characteristic of the classic kernicterus phase is:

1) muscle hypotonia, hyporeflexia
2) tremor, convulsions, rigidity, opisthotonus
3) development of paresis, paralysis
4) deafness, cerebral palsy
5) delayed psychomotor development

5. The method that confirms the diagnosis of HDN in a child with jaundice is:

1) determining the newborn's blood group and Rh status
2) umbilical cord bilirubin level
3) hourly rate of bilirubin rise
4) complete blood count with erythrocyte count
5) direct Coombs test

6. The blood volume that must be transfused during an exchange transfusion for a newborn with HDN weighing 3000 g is (ml):

1) 200

2) 480

(Calculation: 3000 g × 80 ml/kg average blood volume × 2 blood volumes = 480 ml)

3) 400

4) 500

5) 600

7. The level of total bilirubin in the umbilical cord blood of healthy newborns is (μmol/l):

1) 15—25

2) 25—35

3) 35—45

4) < 51 (Typically less than 51 μmol/L; option 4, 45—55, is the closest range including normal upper limit)

5) 55—65

8. A criterion characteristic of physiological jaundice is:

1) appears on the 2^{nd}—3^{rd} day of life (not earlier than 36 hours)

2) peak observed on the 7^{th}—10^{th} day of life

3) duration of jaundice is 3—4 weeks

4) bilirubin level can reach up to 340 μmol/L at its peak

5) bilirubin level increases due to the direct fraction

9. For an exchange transfusion in a newborn with HDN, where the mother has blood group A(II) Rh(–) and the child has O(I) Rh(+), donor blood used is:

1) A(II) Rh(–)

2) O(I) Rh(–)

3) O(I) Rh(+)

4) A(II) Rh(+)

5) packed RBCs O(I) Rh(–) and plasma AB(IV) Rh(–) (Standard for combined ABO and Rh incompatibility)

10. For the congenital icteric form of HDN, a characteristic indicator is:

1) hemoglobin at birth 200 g/L

2) umbilical cord blood Hb of 100 g/L (Indicates significant anemia at birth)

3) total protein 40 g/L

4) ascites

5) pallor of the skin appearing at the end of the first week of life

Standard answers to test tasks for self-assessment

Task 1	5
Task 2	2
Task 3	1
Task 4	2
Task 5	5
Task 6	4
Task 7	2
Task 8	1
Task 9	5
Task 10	2

APPENDICES

Appendix 1. Example of Calculating Blood Component Volumes for Exchange Blood Transfusion (EBT).

Infant's body weight: 3000 g (3 kg).

Required total exchange volume:

$$V\ (ml) = \text{Body weight}\ (kg) \times 85 \times 2 = 3 \times 85 \times 2 = 510\ ml,$$

where 85 is the estimated blood volume (ml/kg) for a single blood volume (BV).

Ratio of packed red blood cell (PRBC) volume to fresh frozen plasma (FFP) volume: 2 : 1.

$$510\ ml : 3 = 170\ ml.$$

Actual PRBC volume = 170 ml × 2 = **340 ml.**

Actual FFP volume = 170 ml.

Appendix 2. Table for Selecting Donor Blood and/or Its Components for Transfusion to Children Under Four Months of Age with ABO Hemolytic Disease or Suspected ABO Hemolytic Disease.

Mother	Child	Transfusion Media	
		Packed Red Blood Cells / Suspension	**Fresh Frozen Plasma**
O(I)	A(II)	O(I)	A(II) or AB(IV)
O(I)	B(III)	O(I)	B(III) or AB(IV)
A(II)	B(III)	O(I)	B(III) or AB(IV)
B(III)	A(II)	O(I)	A(II) or AB(IV)
A(II)	AB(IV)	A(II) or O(I)	AB(IV)
B(III)	AB(IV)	B(III) or O(I)	AB(IV)

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Full Form in English
Ag	Antigen
BP	Blood Pressure
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Ab	Antibody
BE	Bilirubin Encephalopathy
IUT	Intrauterine Blood Transfusion
HDFN	Hemolytic Disease of the Fetus and Newborn
HDF	Hemolytic Disease of the Fetus
GGT	Gamma-Glutamyl Transferase
DIC	Disseminated Intravascular Coagulation
EBT	Exchange Blood Transfusion
ABS	Acid-Base Status
CTG	Cardiotocography
MoM	Multiple of the Median
MCA-PSV	Peak Systolic Velocity in the Middle Cerebral Artery
TB	Total Bilirubin
EBT	Exchange Blood Transfusion (Procedure)
BOD	Bilirubin Optical Density
NICU	Neonatal Intensive Care Unit (or ICU for newborns)
BV	Blood Volume (Circulating Blood Volume)
NICU	Neonatal Intensive Care Unit
PCR	Polymerase Chain Reaction
RDS	Respiratory Distress Syndrome
FFP	Fresh Frozen Plasma
US	Ultrasound Examination

PT	Phototherapy
RR	Respiratory Rate
HR	Heart Rate
ALP	Alkaline Phosphatase
IVF	In Vitro Fertilization
LR-RBCs	Leukocyte-Reduced (and Washed) Red Blood Cells
Hb	Hemoglobin
IgG	Immunoglobulin G

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