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Hemolytic disease of the fetus and newborn: A review of pathophysiology, diagnosis, and management

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ABSTRACT

Hemolytic Disease of the Fetus and Newborn (HDFN) results from maternal alloantibodies attacking fetal red blood cells, leading to fetal anemia and potentially severe complications such as hydrops fetalis. Effective management relies on early detection through maternal antibody screening, fetal antigen testing, and close monitoring of fetal anemia. In cases of severe anemia, intrauterine transfusion (IUT) remains the primary intervention to improve fetal outcomes. Despite this, there is an urgent need for novel medical therapies to prevent or delay disease progression in pregnancies at risk for HDFN.

1. Background

Hemolytic Disease of the Fetus and Newborn (HDFN) is a rare, but serious pregnancy complication that arises when a mother's immune system becomes sensitized to specific antigens on the fetal red blood cells (RBCs), recognizing them as foreign. The alloantibodies produced are primarily of the IgG type which can cross the placenta, bind to fetal RBCs, and mark them for destruction by macrophages in the fetal spleen. The most common antibodies associated with HDFN are those directed against the Rh and Kell blood group antigens [1]. While the Rh system includes over 50 antigens, RhD is most frequently implicated in this condition. The most common antigens, besides RhD, that can lead to HDFN are RhC, Rhc, RhE, Rhe, and Kell. Non-RhD HDFN occurs in approximately 1.5–2.5 % of pregnancies [1]. Among these, the K antigen of the Kell system is particularly concerning. Unlike many other alloantibodies, *anti*-K not only induces hemolysis but also suppresses erythropoiesis by targeting and destroying RBC progenitors and precursor cells in the fetal bone marrow. This dual action can result in an earlier onset of anemia and a more severe clinical course compared to other alloantibodies. A study examining the risk of anemia and dependence on transfusions in severe HDFN identified that 90 % of cases (35/39) of K alloimmunization required intrauterine transfusions (IUT), and 72 % (28/39) of these infants were anemic after birth and needed at least one transfusion [2].

The prevalence of RhD-negative individuals varies across different ethnic groups. According to a study, the Basque population from Spain and France has the highest prevalence, estimated at 30–35 %, while White North Americans or Europeans have around 15 %. Other ethnic groups show lower rates, including 8 % among African Americans, 4–6 % among Africans, and approximately 5 % among Indians [3]. For Native Americans and Inuit populations, the prevalence ranges from 1 to 2 %, while it is much lower in Asian

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populations, generally less than 0.5 % [3].

2. Risk factors

Factors such as miscarriage, pregnancy termination, ectopic pregnancy, bleeding before or during delivery, and certain obstetric procedures (e.g., chorionic villous sampling or amniocentesis) can lead to fetal-maternal blood mixing, thereby increasing the risk of HDFN. A Dutch cohort study involving 113 women exposed to RhD antigen in a prior pregnancy found that certain events were significantly associated with RhD immunization: Cesarean section [OR 1.7 (95 % CI 1.1–2.6)], manual removal of the placenta [OR 4.3 (95 % CI 2.0–9.3)], and postnatal bleeding exceeding 1000 mL [OR 2.0 (95 % CI 1.1–3.6)] [4].

The risk of alloimmunization increases with the volume of fetal blood that enters the maternal circulation. While a small amount (approximately 0.1 mL) typically enters the maternal circulation in nearly all pregnancies, significant fetomaternal bleeding of more than 80 mL, occurs in about 1 in 1000 deliveries [5].

Maternal exposure to foreign RBC antigens can also occur through blood transfusions, especially since donor blood is not routinely screened for antigens beyond ABO and RhD. Blood transfusions are a frequent source of *anti*-K antibodies, which can be particularly concerning for individuals who need regular transfusions, like those with sickle cell disease and thalassemia [6], or activities like needle sharing among intravenous drug users [7].

3. Clinical presentation

The hallmark of HDFN is fetal anemia, defined as hemoglobin levels more than two standard deviations below the mean for gestational age. The severity of anemia is affected by the subclass of antibodies involved. IgG1 and IgG3 are the most effective at causing hemolysis, with IgG1 crossing the placenta earlier and in greater amounts [8]. Additionally, the severity of anemia depends on the efficiency of IgG transport across the placenta and the maturity of the fetal spleen. Genetic variations also play a role, such as polymorphisms affecting Fc receptor function and HLA-related inhibitory antibodies, including the HLA-DQB1 *0201 allele, which is associated with higher levels of *anti*-D antibodies [9].

A severe complication of fetal anemia due to HDFN is hydrops fetalis, leading to the accumulation of extracellular fluid in at least two areas, such as the peritoneal cavity (ascites), pleural cavity (pleural effusion), pericardial cavity (pericardial effusion), or subcutaneous tissue (skin edema). This life-threatening condition is often characterized by hemoglobin levels more than 7 g/dL below the mean for gestational age and a hematocrit below 25 % [8]. In addition to fluid accumulation, hydropic fetuses may also develop thrombocytopenia and neutropenia. A systematic review on HDFN found that hydrops fetalis was the most common outcome across all studies, with 14.8 % of Rh-D mediated hydrops cases requiring transfusions [10]. Severe anemia can present as hyperbilirubinemia postnatally, increasing the risk of kernicterus, a severe neurological condition that can result in permanent brain damage or death [11].

4. Screening and primary prevention

Screening for alloimmunization is an essential aspect of antenatal care. At the first prenatal visit, RhD typing, and an antibody screen are conducted to identify pregnancies at risk. To prevent RhD alloimmunization, polyclonal *anti*-D immunoglobulin (RhIg) is universally recommended for all non-sensitized RhD-negative women. According to the American College of Obstetricians and Gynecologists (ACOG) guidelines, antenatal prophylaxis is advised at 28 weeks of gestation, while postnatal prophylaxis should be administered immediately after the delivery of an RhD-positive neonate, ideally within 72 h. RhIg is also recommended following any event that could lead to fetal-maternal blood mixing. This includes pregnancy loss or termination, ectopic and molar pregnancies, trauma, or invasive procedures such as amniocentesis, chorionic villus sampling, fetal blood sampling, and fetal intervention procedures, all of which carry a risk of fetal-maternal hemorrhage [12].

While polyclonal *anti*-D immunoglobulin has been highly effective in reducing RhD alloimmunization, it has not eliminated the disease. Although preliminary findings suggest that monoclonal RhIg might be a useful alternative to polyclonal RhIg, more extensive and rigorous trials are necessary to establish its efficacy [13].

In high-income countries, adherence to RhIg protocols is generally high, but many developing countries still face significant challenges. For example, an estimated 1,000,000 RhD-negative women in Pakistan and India do not receive *anti*-D prophylaxis, resulting in over 100,000 cases of RhD disease annually [3]. It has also been showed that almost 50 % of the women around the world who require this type of prophylaxis do not receive it, presumably due to a lack of awareness, availability, and/or affordability [14]. Additionally, the absence of immunoglobulins to prevent HDFN caused by antibodies other than RhD further complicates efforts to achieve total prevention.

5. Antenatal screening – secondary prevention

If the antibody screen is positive or there is an obstetric history indicating risk for HDFN, further workup is indicated to estimate the risk of HDFN in current pregnancy. In such cases, paternal antigen typing can be an initial step to evaluate the risk. If the father is negative for the given antigen, the fetus is not at risk and can proceed to routine care. If the father is homozygous for the given antigen, further fetal testing is unnecessary as the fetus is assumed to be positive. However, if the father is heterozygous or his antigen status is unknown, further fetal testing is required to confirm the fetus's antigen status.

Traditionally, amniocentesis has been the standard method to determine the fetal RhD status when there is a risk of HDFN. More

recently, non-invasive testing using cell-free fetal DNA (cfDNA) is being performed. This method involves analyzing fetal DNA circulating in the maternal bloodstream and can typically be performed after 11 weeks of gestation. In the United States, one laboratory-sponsored peer-reviewed publication presents minimal data to support the validity and utility of a novel and proprietary technology; clinical validation included 23 RhD-negative pregnant individuals [15]. A second prospective cohort study from this same laboratory recently reported the detection of RhD and other red blood cell antigens comparing results of cfDNA analysis and neonatal genotyping based on samples from 155 alloimmunized pregnancies and neonates, demonstrating 100 % concordance among samples with RhD antigen calls [16]. "No call" results were excluded from analysis, a factor that potentially impacts test performance and should be explored in future studies. A March 2024 ACOG Practice Advisory *Rho(D) Immune Globulin Shortages* suggests that using cfDNA to determine fetal RhD status to prioritize and conserve RhIg is a "reasonable consideration in the practice setting that is experiencing RhIg shortages [17]."

A critical antibody titer, generally defined as 1:16 for non-Kell antibodies and 1:4 for Kell antibodies, serves as a threshold for more intensive monitoring or intervention [1]. For pregnancies where antibody titers exceed the critical threshold, or those with a history of a previous fetus affected by HDFN that required therapy, or a fetal death due to HDFN, noninvasive monitoring using Middle Cerebral Artery peak systolic velocity (MCA-PSV) Doppler ultrasound is indicated [8]. MCA-PSV increases as the fetal hemoglobin drops.

In pregnancies previously affected by HDFN requiring therapy, MCA-PSV monitoring generally begins at 16 weeks of gestation. A meta-analysis that evaluated the diagnostic accuracy of MCS-PSV, found that using trends in MCA-PSV measurements, rather than a single value, improves the accuracy of detecting severe fetal anemia, reducing the false-positive rate to less than 5 % [18].

The ideal frequency for Doppler examinations has not been established. However, experts recommend one to two-week intervals, guided by clinical experience and existing knowledge about the progression of fetal anemia in these cases [18]. An MCA-PSV measurement greater than 1.5 MoM is used as a cut-off for detecting severe fetal anemia, while also evaluating other sonographic findings such as hydrops, cardiomegaly, polyhydramnios, and others. If the MCA-PSV stays below 1.5 MoM, delivery can be scheduled between 37 + 0 and 38 + 6 weeks, following the Society for Maternal-Fetal Medicine guidelines and the American College of Obstetricians and Gynecologists guidelines [1,8]. This strategy is advised because the ability of MCA-PSV to detect severe anemia diminishes after 35 weeks, making the benefits of delivery and subsequent neonatal evaluation outweigh the minor risks associated with early-term birth.

6. Management of severe fetal anemia

- ***Intrauterine transfusion***

IUT is a life-saving procedure where RBCs are directly infused into the fetal circulation to treat severe anemia. First introduced in the 1960s, IUT has been the standard treatment for managing severe fetal anemia for decades [19,20]. The success of IUT depends on the meticulous preparation of the blood used for transfusion and to be done under experienced hands. Typically, type O RhD-negative blood is selected to minimize the risk of antigen incompatibility. The blood is tightly packed to achieve a high hematocrit level (75–85 %), ensuring sufficient oxygen delivery while minimizing the volume transfused, thereby reducing strain on the fetal circulation. To further enhance safety, the blood is irradiated and leuko-depleted, decreasing the risk of complications such as graft-versus-host disease [20].

- ***Approach***

Intravascular transfusion (IVT) is the most common and preferred approach, involving the direct transfusion of RBCs into the fetal circulation through the umbilical vein, under ultrasound guidance. This method is particularly suited for managing severe fetal anemia when rapid correction of hemoglobin levels is required.

IUT is generally performed between 18 and 35 weeks of gestation, with timing critical to achieving the best outcomes. Early IUT, conducted between 18 and 24 weeks, is often required for severely anemic fetuses but carries a higher risk of complications. A study by Yinon et al. reported a 20 % risk of fetal loss when performed before 22 weeks [19], and another study by Zwiers et al. reported an 8 %–16 % procedure-related fetal death rate when performed before 20 weeks, compared to just 0.9 % when done after 20 weeks [21]. The goal is to achieve a target fetal hemoglobin level of 14–16 g/dL or a hematocrit of 40–45 %. Intraperitoneal transfusion (IPT) involves infusing RBCs into the fetal peritoneal cavity, where they are gradually absorbed into the circulation. This approach is typically reserved for situations where intravascular access is not feasible or carries a high risk, particularly in earlier gestations. Intraperitoneal transfusions often begin between 15 and 20 weeks, starting with smaller volumes (5 mL at 15 weeks) and gradually increasing to 10–20 mL by 20 weeks. Repeat transfusions are scheduled at 2–3-week intervals (sometimes even sooner such as in hydrops) until the fetus reaches a gestational age (usually around 20–22 weeks) when intravascular transfusion can be safely performed [22,23]. Although less invasive, IPT corrects fetal anemia more slowly, making it less suitable for severe cases. However, it offers a safer alternative for early intervention or when direct intravascular transfusion is not an option, helping to maintain fetal hemoglobin and hematocrit levels within a safe range until the fetus is more mature or can undergo intravascular transfusion.

- ***Monitoring and Follow-Up***

Subsequent transfusions are guided by weekly MCA-PSV measurements and the expected decline in fetal hemoglobin—approximately 0.9 g/dL per day for the first 10–14 days after the initial transfusion, and about 0.6 g/dL per day over the following 2–3 weeks [1,8]. Transfusions should be individualized, considering the underlying pathology, fetal condition, and post-transfusion

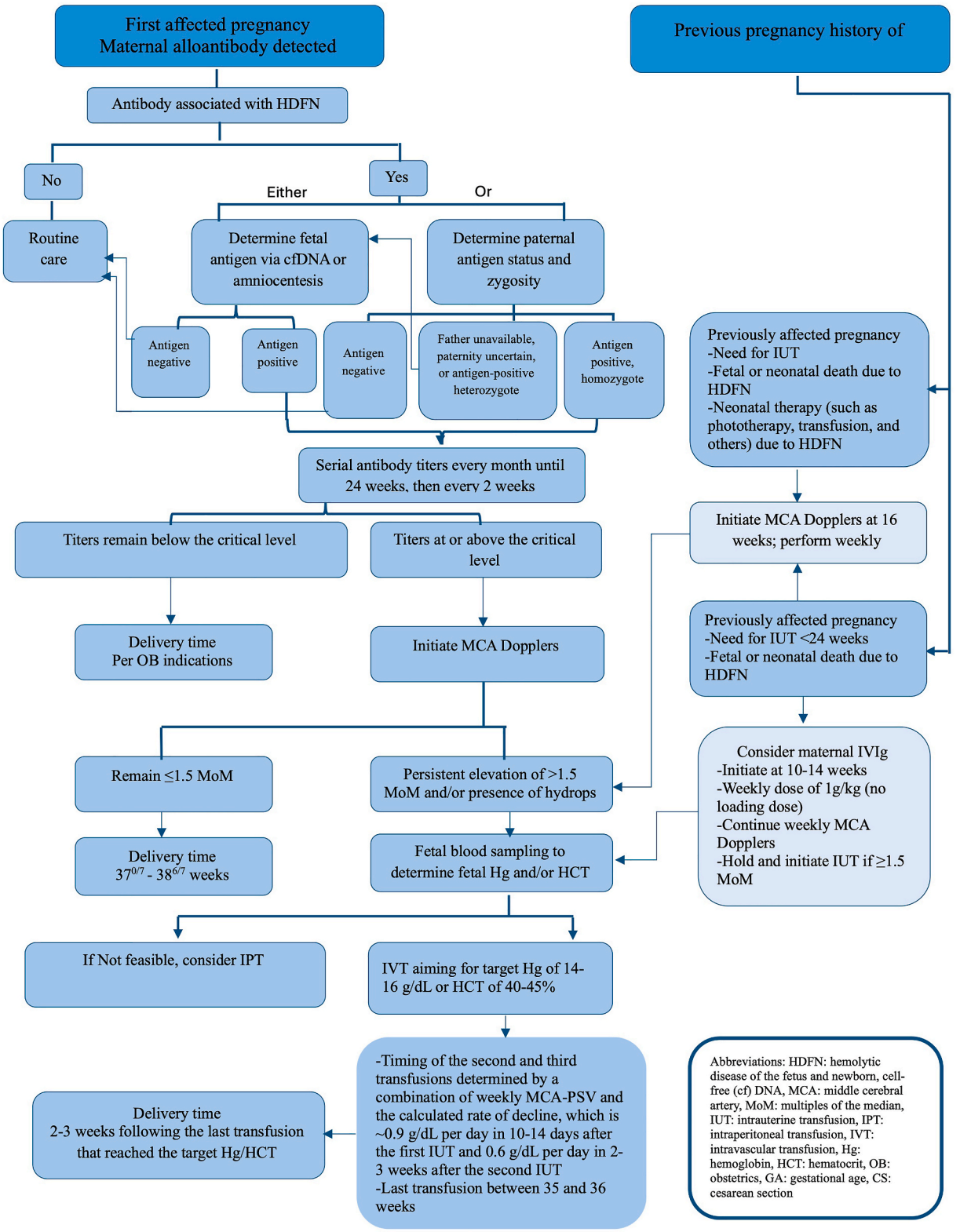

Fig. 1. Recommended approach to screening, monitoring, and management of pregnancies with HDFN.

1. Serial antibody titers and middle cerebral artery doppler scans help assess fetal anemia and guide timely intervention.
2. Intrauterine transfusion remains the gold standard for treating severe fetal anemia. It should be performed by specialists to ensure the best possible fetal outcomes.
3. IVIg can delay the need for IUT by modulating the maternal immune response and limiting antibody transport across the placenta.
4. Nipocalimab, an FcRn receptor blocker, has demonstrated potential in phase 2 trials by reducing the need for early IUT in severe HDFN, and providing a non-surgical management alternative.

1. Future studies should focus on the long-term developmental and health outcomes of fetuses exposed to IVIg during pregnancy.
2. A Phase 3 trial of Nipocalimab is needed to determine its optimal dosing and safety profile.
3. Research should compare the cost-effectiveness of IVIg versus Nipocalimab.
4. There is insufficient data on IVIg usage in developing countries. Future research should explore the effectiveness, accessibility, and fetal outcomes of IVIg therapy in these settings.

hematocrit levels, rather than relying solely on MCA-PSV measurements.

Experts generally recommend planning delivery between two to three weeks following the last transfusion to balance the risks of stillbirth, ongoing complications from fetal anemia, and the potential dangers of further fetal blood sampling or transfusions against the risks of prematurity and additional morbidity from anemia or hyperbilirubinemia. Consequently, most clinicians aim to perform the final fetal blood sampling and transfusion by 35 weeks.

7. Workflow of screening, monitoring, and management

The authors of this review included a workflow that can be utilized as guidance for the screening, monitoring, and management of pregnancies with HDFN as shown in Fig. 1.

7.1. HDFN in the neonatal period

In the neonatal period, major clinical concerns include anemia and hyperbilirubinemia, which, if severe, can lead to kernicterus [24]. Newborns need to be monitored for up to three months of age as per consensus of experts in an international Delphi study on HDFN management [25]. Management of affected newborns often requires intensive interventions such as phototherapy, blood product transfusions, and exchange transfusion. Additionally, infants who received in utero transfusions may be at higher risk of developing late-onset anemia, necessitating further transfusions [26].

8. Summary

In summary, HDFN occurs when maternal alloantibodies target fetal red blood cells, leading to fetal anemia and complications like hydrops fetalis. Management involves early detection through maternal antibody screening, fetal antigen testing, and monitoring fetal anemia. IUT is a life-saving procedure for pregnancies complicated by severe fetal anemia due to alloimmunization. However, there is a critical need for medical therapies that can prevent or delay the progression of fetal anemia, reducing the necessity for IUT in high-risk pregnancies.

Practice points

Research agenda

CRediT authorship contribution statement

Hajra Malik: Writing – review & editing, Writing – original draft. **E.J.T. (Joanne) Verweij:** Writing – review & editing, Writing – original draft. **Hiba J. Mustafa:** Writing – review & editing, Supervision, Visualization, Conceptualization, Writing – original draft.

Conflicts of interest

The authors report no conflicts of interest.

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References

[1] ACOG Practice Bulletin No. 192: management of alloimmunization during pregnancy. Obstet Gynecol 2018;131:e82–90. https://doi.org/10.1097/aog.0000000000002528.
[2] Ree IMC, et al. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the fetus and newborn in the first 3 months after birth. Br J Haematol 2019;186:565–73. https://doi.org/10.1111/bjh.15962.
[3] Zipursky A, Paul VK. The global burden of Rh disease. Arch Dis Child Fetal Neonatal Ed 2011;96:F84–5. https://doi.org/10.1136/adc.2009.181172.
[4] Slootweg YM, et al. Risk factors for RhD immunisation in a high coverage prevention programme of antenatal and postnatal RhIg: a nationwide cohort study. BJOG 2022;129:1721–30. https://doi.org/10.1111/1471-0528.17118.
[5] Medearis AL, Hensleigh PA, Parks DR, Herzenberg LA. Detection of fetal erythrocytes in maternal blood post partum with the fluorescence-activated cell sorter. Am J Obstet Gynecol 1984;148:290–5. https://doi.org/10.1016/s0002-9378(84)80070-8.
[6] Davoudi-Kiakalayeh A, Mohammadi R, Pourfathollah AA, Siery Z, Davoudi-Kiakalayeh S. Alloimmunization in thalassemia patients: New insight for healthcare. Int J Prev Med 2017;8:101. https://doi.org/10.4103/ijpvm.IJPVM_246_16.
[7] Lappen JR, Stark S, Gibson KS, Prasad M, Bailit JL. Intravenous drug use is associated with alloimmunization in pregnancy. Am J Obstet Gynecol 2016;215(344):e341–6. https://doi.org/10.1016/j.ajog.2016.03.019.
[8] Mari G, et al. Society for Maternal-Fetal Medicine (SMFM) clinical guideline #8: the fetus at risk for anemia–diagnosis and management. Am J Obstet Gynecol 2015;212:697–710. https://doi.org/10.1016/j.ajog.2015.01.059.
[9] Hildén JO, Gottvall T, Lindblom B. HLA phenotypes and severe Rh(D) immunization. Tissue Antigens 1995;46:313–5. https://doi.org/10.1111/j.1399-0039.1995.tb02498.x.
[10] de Winter DP, Kaminski A, Tjoa ML, Oepkes D. Hemolytic disease of the fetus and newborn: systematic literature review of the antenatal landscape. BMC Pregnancy Childbirth 2023;23:12. https://doi.org/10.1186/s12884-022-05329-z.
[11] Bhutani VK, et al. Neonatal hyperbilirubinemia and rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res 2013;74(Suppl 1):86–100. https://doi.org/10.1038/pr.2013.208.
[12] Practice bulletin no. 181: prevention of Rh D alloimmunization. Obstet Gynecol 2017;130:e57–70. https://doi.org/10.1097/aog.0000000000002232.
[13] Verweij E, et al. Monoclonal RhD prophylaxis: high time to evaluate efficacy. Lancet 2024;403:806–7. https://doi.org/10.1016/s0140-6736(23)01888-3.
[14] Pegoraro V, et al. Hemolytic disease of the fetus and newborn due to Rh(D) incompatibility: a preventable disease that still produces significant morbidity and mortality in children. PLoS One 2020;15:e0235807. https://doi.org/10.1371/journal.pone.0235807.
[15] Alford B, et al. Validation of a non-invasive prenatal test for fetal RhD, C, C, E, K and Fy(a) antigens. Sci Rep 2023;13:12786. https://doi.org/10.1038/s41598-023-39283-3.
[16] Rego S, et al. Cell-Free DNA analysis for the determination of fetal red blood cell antigen genotype in individuals with alloimmunized pregnancies. Obstet Gynecol 2024. https://doi.org/10.1097/aog.0000000000005692.
[17] American College of Obstetricians and Gynecologists *Rho(D*. Immune globulin shortages. https://www.acog.org/clinical/clinical-guidance/practice-advisory/articles/2024/03/rhod-immune-globulin-shortages; 2024.
[18] Pretlove SJ, Fox CE, Khan KS, Kilby MD. Noninvasive methods of detecting fetal anaemia: a systematic review and meta-analysis. BJOG 2009;116:1558–67. https://doi.org/10.1111/j.1471-0528.2009.02255.x.
[19] Moise Jr KJ, Argoti PS. Management and prevention of red cell alloimmunization in pregnancy: a systematic review. Obstet Gynecol 2012;120:1132–9. https://doi.org/10.1097/aog.0b013e31826d7dc1.
[20] Pasman SA, et al. Intrauterine transfusion for fetal anemia due to red blood cell alloimmunization: 14 years experience in Leuven. Facts Views Vis Obgyn 2015;7:129–36.
[21] Zwiers C, et al. Complications of intrauterine intravascular blood transfusion: lessons learned after 1678 procedures. Ultrasound Obstet Gynecol 2017;50:180–6. https://doi.org/10.1002/uog.17319.
[22] Watts DH, et al. Intraperitoneal fetal transfusion under direct ultrasound guidance. Obstet Gynecol 1988;71:84–8.
[23] Fox C, Martin W, Somerset DA, Thompson PJ, Kilby MD. Early intraperitoneal transfusion and adjuvant maternal immunoglobulin therapy in the treatment of severe red cell alloimmunization prior to fetal intravascular transfusion. Fetal Diagn Ther 2008;23:159–63. https://doi.org/10.1159/000111599.
[24] Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. Expert Rev Hematol 2017;10:607–16. https://doi.org/10.1080/17474086.2017.1331124.
[25] Mustafa HJ, Sambatur EV, Shamshirsaz AA, Johnson S, Moise KJ, Baschat AA, et al. Monitoring and management of hemolytic disease of the fetus and newborn based on an international expert Delphi consensus. Am J Obstet Gynecol 2024;232:280–300. https://doi.org/10.1016/j.ajog.2024.11.003.
[26] Tyndall C, Cuzzilla R, Kane SC. The rhesus incompatible pregnancy and its consequences for affected fetuses and neonates. Transfus Apher Sci 2020;59. https://doi.org/10.1016/J.TRANSCI.2020.102948.