Bridging the gaps: prevention, management, and future perspectives in hemolytic disease of the fetus and newborn

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Identification and management of fetal anemia due to hemolytic disease

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Abstract

Introduction: Hemolytic disease of the fetus and newborn (HDFN) is a condition caused by maternal alloantibodies against fetal red blood cells (RBCs) that can cause severe morbidity and mortality in the fetus and newborn. Adequate screening programs allow for timely prevention and intervention resulting in significant reduction of the disease over the last decades. Nevertheless, HDFN still occurs and with current treatment having reached an optimum, focus shifts towards non-invasive therapy options.

Areas covered: This review focusses on the timely identification of high risk cases and antenatal management. Furthermore, we elaborate on future perspectives including improvement of screening, identification of high risk cases and promising treatment options.

Expert opinion: In high-income countries mortality and morbidity rates due to HDFN have drastically been reduced over the last decades, yet worldwide anti-D mediated HDFN still accounts for 160.000 perinatal deaths and 100.000 patients with disabilities every year. Much of these deaths and disabilities could have been avoided with proper identification and prophylaxis. By implementing sustainable prevention, screening and disease treatment measures in all countries this will systemically reduce unnecessary perinatal deaths. There is a common responsibility to engage in this cause.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is a disease caused by maternal alloantibodies of the IgG class against fetal red blood cell (RBC) antigens. During pregnancy, if the fetus is positive for the implicated antigen, antibodies can bind to the RBCs and to erythroid progenitors. This binding leads to destruction of fetal RBCs (hemolysis). If RBC alloantibodies bind to early erythroid progenitors, this may lead to impairment of the erythropoiesis and early anemia in the fetus. If fetal anemia is left untreated this can lead to cardiomegaly, fetal hydrops, and even death. After birth, ongoing hemolysis can cause severe hyperbilirubinemia, and cause damage to the basal ganglia leading to a condition called kernicterus. The range of neurological disorders in HDFN varies from minor disabilities to deafness and severe irreversible damage of the central nervous system.

Most of the severe cases of HDFN are caused by alloimmunization against the D antigen, part of the Rh blood group system. Other contributors are antibodies targeting the K antigen (of the Kell blood group system) and c and other Rh antigens. Severe HDFN is seldom caused by other antibodies.[1,2] In the years following the discovery of the cause of HDFN, numerous strategies were introduced to reduce the occurrence of the disease. One of the most beneficial strategies is RhD immunoprophylaxis (RhIg) to prevent formation of alloantibodies against the D antigen. In most high- and medium-income countries, RBC D typing and screening for RBC antibodies is conducted routinely early in pregnancy and in the third trimester. During pregnancy and after the birth of a D-positive child, RhD-negative women are protected against D-immunization by RhIg.[3-7]

When alloantibodies are detected, the severity of HDFN is commonly evaluated by assessing the antibody titer. Alternatively, or in addition, antibody quantitation or a bioassay such as the antibody/dependent cellular cytotoxicity (ADCC) can be used. For each test cutoff levels have been determined to guide further management. A titer of 16 is generally considered the cutoff for referral to a center of expertise in maternal-fetal medicine.[8,9] Pregnancies considered high-risk by serologic testing are managed by serial (often weekly) ultrasound and Doppler examinations aimed to detect fetal anemia requiring intervention. The best predictor for fetal anemia is Doppler assessment of the peak systolic velocity in the middle cerebral artery (PSV-MCA).[10,11] A blood flow velocity above the generally accepted cutoff level of 1.5 MoM (multiple of the mean) accurately predicts the presence of severe anemia, which can be treated either by intrauterine blood transfusion (IUT) or by delivery followed by transfusion in the neonate. In 1963, Liley described the first intraperitoneal blood transfusion[12] paving the way for the nowadays successful, and in experienced hands safe, ultrasound guided intravascular intrauterine transfusions. These innovations improved fetal outcome significantly.[13]

Since the discovery of anti-D-mediated HDFN in the 1930s and 40s by a combined effort from various researchers and research groups, management has greatly improved making it a rare disease and reducing perinatal mortality drastically by timely intervention.[14,15] Bowman wrote an article going more into depth as to the history of the discovery of the disease and the development of the management.[15]

Despite all these improvements, HDFN has not yet been eradicated. Alloimmunization in RhD-negative women still has an estimated incidence of 0.3-1.3%.[16] Besides this, prophylaxis programs do not exist for other RBC antigens, which allows for continued alloimmunization due to non-D antigens.[2] Furthermore, identification of high-risk cases is still challenging: antibody titers representing antibody levels in the mother are not perfect for identifying pregnancies at risk for severe HDFN. The generally considered cutoff of 16 has a false positive rate of 77% for predicting fetal anemia.[8] Additionally, although IUTs are nowadays considered to be a safe procedure, procedure-related fetal loss still ranges from 1.8%-4.7% per fetus and 0.6%-1.4% per procedure.[13,17-19] In early gestation, between 16 and 20 weeks, fetal loss rates are even up to 20%.[20] With improvements in IUT treatment reaching an optimum, the focus shifts to noninvasive treatment options to prevent or treat severe HDFN.

This review focusses on the timely identification of high-risk cases and antenatal treatment management, and we elaborate on future perspectives to improve screening, identification of high-risk cases, and promising treatment options.

Identification of cases 'at-risk' for severe fetal anemia in HDFN

Adequate early identification of pregnancies 'at-risk' is essential for efficient management of HDFN.

Clinical relevance of RBC alloantibodies

Over 400 different RBC antigens are identified. Not all of them are known to be a cause of HDFN. Whether an RBC antigen and the corresponding maternal alloantibody should be regarded as a potential risk to cause HDFN depends on multiple factors: the level of expression of the RBC antigen on the fetal RBCs, the Ig subclass of the alloantibodies, the alloantibody concentration and characteristics of the IgG-Fc tail that may influence the binding to IgG-Fc receptors.[21,22]

The D antigen is the most immunogenic of all RBC antigens. RhD-negative women have an immunization rate of around 16% in a next pregnancy, when no RhIg has been

given in the fore-going pregnancy of a RhD-positive child.[23] In the Netherlands, the RhIg program resulted in an immunization prevalence of 0.63%,[16] which is in accordance with what is found in other studies.[16] In this study, a risk factor for immunization in presence of RhIg was found to be a complicated delivery, e.g. cesarean section, major postpartum hemorrhage and manual placental removal.[16]

Other RBC alloantibodies contributing to occurrence of severe HDFN are anti-c and anti-e (both Rh type antibodies) and anti-K (Kell). The latter has the highest risk of inducing severe HDFN with already early occurring fetal anemia, because of binding to erythroid progenitor cells.[24] Other specificities of RBC alloantibodies that may interfere with erythropoiesis are anti-M of IgG subclass and anti-Ge antibodies, recognizing antigens of the MNS blood group system. The risk to develop severe HDFN for certain type of antibodies (e.g. anti-M) may differ in the various populations.[25-28] We previously described the clinical relevance of RBC alloantibodies in HDFN and summarized them.[1]

Screening for alloantibodies

Programs

In most high-resource countries a program is set up to type for the D antigen and to screen for RBC alloantibodies early in pregnancy.[1,2,4-7,29,30] Because of the increasing risk of fetomaternal hemorrhage (FMH) during pregnancy, primary alloimmunization, or a boostering of a previous alloimmune response, can occur during pregnancy. In these cases, an RBC alloantibody may only become detectable later in pregnancy. Therefore, screening for RBC alloantibodies is repeated in the third trimester in most countries; in all women or in a subgroup, such as D-negative women.[4,31]

RBC alloantibody identification and quantification

The antibody titer aims to predict severe RBC hemolysis in the fetus. Though not precise, it can be used for the preselection of cases for follow up via ultrasound assessment. Titers are repeated at regular intervals up until a so-called 'critical titer' or 'cutoff' level is reached and referral to a center of expertise in maternal-fetal medicine is advised. When comparing titers, it is important that comparable techniques are applied. For instance, the use of single-dose antigen positive or double-dose antigen-positive RBCs is of significance. In the Netherlands double-dose antigen-positive RBCs are used for Rh-antibodies (c, D and E), for all other antibodies single-dose antigen positive RBCs are used.[32] The cutoff value for anti-D lies between 8-32, depending on the techniques used in the laboratory,[9,33] for anti-K this cutoff is usually much lower as it has lower predictive

value and may cause early severe fetal anemia with low antibody titers.[4,34] The British guideline of the Royal College of Obstetricians and Gynecologists proposes to use a cutoff of 8[4] for anti-K and recently Slootweg et al. presented that a titer of 4 had a sensitivity of 100% to detect severe HDFN, although the specificity was only 36%.[34] Both sources indicate that anti-K can cause HDFN at very low titers. For all other RBC alloantibodies in general a cutoff of 16 or 32 is used.[1,2,5,30,31] Quantitative assays have also been developed to determine the antibody concentration with more accuracy compared to the titration method. One of these techniques was the AutoAnalyzer technique.[35] Though promising, after comparison it had only a marginally better predictive value.[36]

Bioassays

As previously mentioned, the ability of alloantibodies to cause HDFN is multifactorial. The amount of alloantibodies alone is not specific enough to determine the risk for HDFN. To determine the ability of RBC alloantibodies to initiate interaction with phagocytes various cellular assays have been developed. They are based on interactions with either monocytes or NK cells. Here, we discuss bioassays that have been developed as predictors for HDFN, and their limitations.

Antibody-dependent cellular cytotoxicity

Two types of antibody-dependent cellular cytotoxicity (ADCC) assays were developed.[35] In general, in such assays, RBCs positive for a predefined set of RBC antigens are labelled with 51-sodium chromate and incubated with maternal serum containing RBC alloantibodies and mononuclear cells (e.g. either monocytes or lymphocytes, containing IgG-Fc receptor type III (CD16) positive lymphocytes: a subset of NK cells). As the RBCs lyse, the ^{51}Cr is released and is measured by a gamma counter. The level of released ^{51}Cr is then used as a measure for hemolytic activity. The NK-cell based ADCC quantifies extra-cellular hemolysis by lymphocytes and was the first cellular test to be developed as a predictor for HDFN.[37] The assay was tested in relatively small series of samples by Urbaniak et al.[37] who found a good predictive value though Hadley et al.[38] did not confirm this.

Monocytes were also used in a similar set-up. Numerous studies evaluated the predictive value of the monocyte-based ADCC and found it to be more predictive for disease severity compared to the indirect antiglobulin test (IAT).[39] Oepkes et al.[8,32] showed that combined with the antibody titers it improves diagnostic accuracy for prediction of high-risk cases, both for D and non-D antibodies.[8,32] Therefore, this assay was implemented

in the laboratory work up for detection of high risk cases in the Netherlands.[1,5] However, the use of radioactive chromium makes implementation of the ADCC in most reference laboratories not amenable.

Chemiluminescence test

When sensitized RBCs adhere to monocytes and phagocytosis occurs, oxygen radicals are freed. This process can be visualized when using luminol at incubation, which reacts with the oxygen radicals and produces light which can be measured in a luminometer.[35] In multiple studies the results from this chemiluminescence test (CLT) had higher predictive value for fetal outcomes compared to the AutoAnalyzer, though it was less safe relying on negative results. It seems to be suitable to distinguish women with high levels of antibodies that generally invoke a mild disease severity, but not as negative predictor.[40,41]

Monocyte monolayer assay

Another monocyte-based bioassay is the monocyte monolayer assay (MMA). This assay determines the level of phagocytosis and adherence of sensitized RBCs to monocytes.[35] This is examined microscopically after incubation. The additional predictive value of MMA over manual titration has been proved in some studies but not confirmed in others.[42,43] Moreover, in multiple studies the ADCC and CTL were superior in predicting HDFN.[41-44]

Fetal antigen typing

Paternal phenotyping

If clinically relevant RBC alloantibodies are detected in maternal plasma it can be useful to determine the phenotype of paternal antigens. If the father is homozygous for the implicated antigen, the fetus will automatically be at risk for HDFN. The reverse is also true, if the father is negative the fetus will not be at risk and further testing is irrelevant.[45] Evidently, certainty as to paternity is crucial when typing paternal antigens.

Noninvasive fetal phenotyping

If the paternal phenotype is heterozygous, or if there is uncertainty about paternity, the phenotype of the fetus should be established. This used to be an invasive procedure where fetal material was collected by amniocentesis or chorionic villi sampling. For D, C, c, E and K this can be done noninvasively by amplifying cell-free fetal DNA (cffDNA) from

maternal plasma using various PCR based assays.[46] The sensitivity and specificity of these noninvasive techniques have been extensively studied and gives sensitivity levels of 99-99.9% and specificity of 95% or higher.[47] The noninvasiveness of these techniques reduced miscarriage rates and mild-to-severe hemolysis conversions associated with more invasive techniques.[48]

Laboratory management to prevent D alloimmunization and timely detect cases 'at-risk'

Laboratory management should focus on optimal prevention of D alloimmunization in D-negative women and timely administration of RhIg, with additional doses in cases of suspected FMH.[4-7] The follow-up with repeat titer measurements, and in some cases bioassays such as the ADCC may be limited to pregnancies with Rh and K antibodies. For Rh, K and all other type of RBC alloantibodies it is of equal importance to be aware of the presence of RBC alloantibodies in the mother, independent of the titers. (Neonatal) caregivers should anticipate the possibility of hyperbilirubinemia due to the alloantibodies and systematic policies should be in place to asses for this occurrence. The flow chart in Figure 1 sets out the complete antibody screening and antigen typing done in pregnancy.

Antenatal treatment HDFN

Monitoring severe HDFN

When laboratory results indicate an increased risk for HDFN weekly ultrasounds are indicated to determine the risk of fetal anemia. Timely treatment of anemia is important to prevent hydrops fetalis or fetal death. It is known that hydrops fetalis is a risk factor for worse/adverse short- and long-term outcomes.[49,50]

Historically, determination of the severity of anemia was done by measuring bilirubin levels in amniotic fluid obtained by amniocentesis also known as the Liley's method. The bilirubin levels reflect the extent of hemolysis well.[51] Around the 1990s innovations in noninvasive Doppler ultrasound techniques improved and enabled obstetric care providers to measure blood flow in the fetus.[11] The peak systolic velocity in the middle cerebral artery (MCA-PSV) is increased in anemic fetuses due to the increased cardiac output and reduced blood viscosity. The middle cerebral artery was selected due to its rapid response to hypoxemia, easy visualization, and low intraobserver and interobserver variability.[52] Weekly measurements of the MCA-PSV are the current gold standard screening tool for fetal anemia.[10,53,54] Reference values were set up by Mari et al.[54] in 2000 finding and optimal cut off for severe anemia at 1.55 multiple of the median (MoM) with 100% sensitivity and a false positive rate of 12%.[54] Furthermore, the fetus is

checked for other characteristics of anemia such as cardiomegaly, increased diameter of the umbilical vein, enlarged spleen or increased opacity in the bowel.[55-57] Both the reference values of Mari et al.[54] as these other sonographic factors determine the need for fetal blood sampling to definitively determine fetal hemoglobin levels and perform IUT. Sole diagnostic blood sampling without transfusion is not recommended.[58]

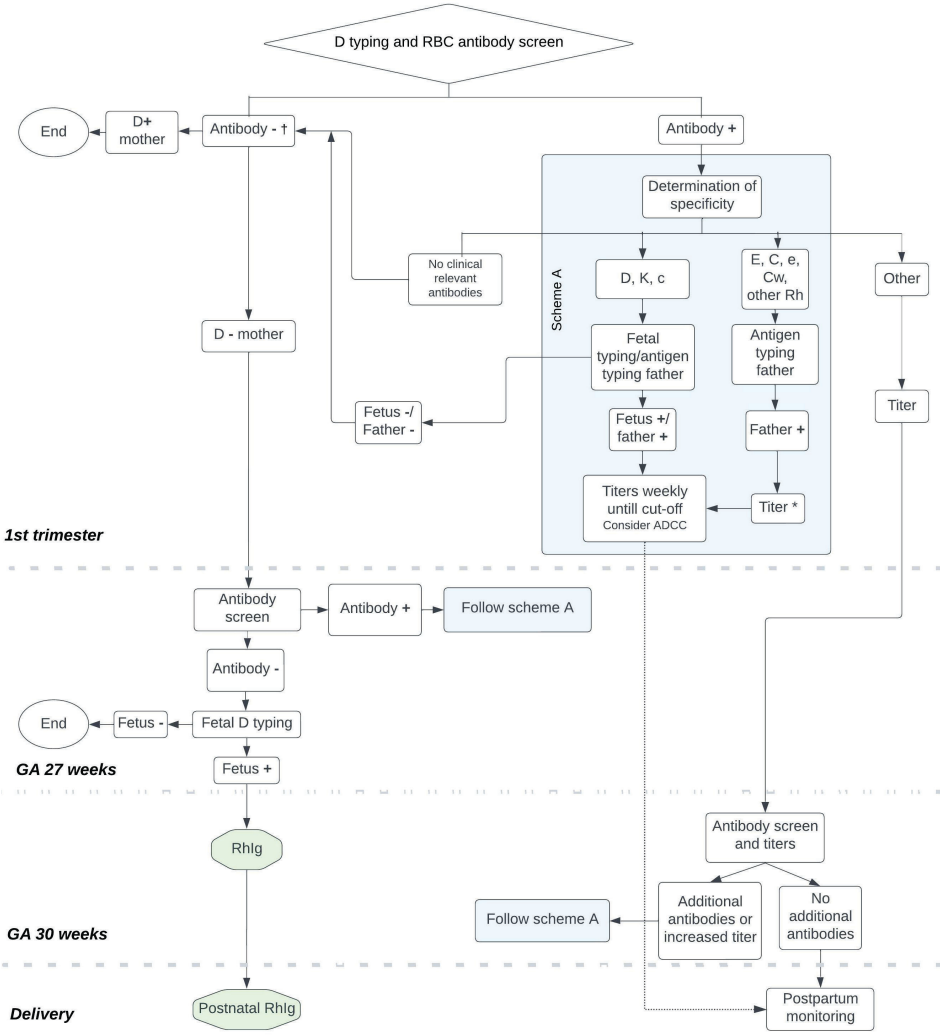

Figure 1. Red cell antibody screening and red cell antigen typing for timely management of HDFN. In green the administration of RhIg is depicted. In blue 'Scheme A' which refers to the management of antibodies relevant for HDFN. When 'cut off' is reached, referral to a center of expertise in maternal-fetal medicine is advised.

***** If titers are ≥1:16, consider fetal antigen typing and follow up to weekly titers and/or ADCC. Otherwise follow the flowchart from 'other' onwards. † Antibodies of no clinical significance to cause HDFN. RBC: red blood cell. RhIg: RhD immunoprophylaxis. HDFN: hemolytic disease of the fetus and newborn.

Intrauterine transfusions

Intravascular transfusions

IUTs have since its originating in 1981 developed into the cornerstone of treatment for fetal anemia.[58] Once starting as intraperitoneal transfusions, this technique developed into ultrasound-guided intravascular transfusion in the umbilical cord or intrahepatic vein. Zwiers et al. previously extensively described the setting and technical details in a review in this journal in 2017.[58] Currently, this procedure is relatively safe when performed in an experienced center with survival rates varying form 88.9-100%.[58] This safety is reflected in the procedure related complication and fetal loss. Tiblad et al. reported a procedure related fetal loss rate of 4.7% per fetus, 1.4% per procedure in a cohort of patients between 1990 and 2010 (n=284).[19] Pasman et al. found no fetal demise and severe adverse events in 1.5% of the procedures performed between 2000 and 2014 (n=135).[17] In our center we found similar results with procedure related complications of 1.3% per procedure and procedure related fetal demise of 1.8% per fetus (n=937).[13] Some factors contributing to fewer procedure related complications and thus improved fetal and neonatal outcome are routine use of fetal paralysis, increased use of the intrahepatic route and avoidance of arterial puncture and transamniotic 'free loop' needling.[13,17,19]

Complications

As successful as this therapy is, there are some drawbacks to be aware of. Though IUTs are perceived as relatively safe, earlier gestational age carries a higher risk for complications and fetal loss. This can be explained by the size of the umbilical vein being 3-5 mm at gestation before 20 weeks making the procedure technically difficult. In addition, early severe cases progress rapidly resulting in more severe anemia and hydrops at first IUT.[57] Yinon et al. described a cohort of 30 pregnancies with early severe RBC alloimmunization requiring first IUT before 22 weeks of gestation. They found perinatal survival of 80% and procedure related fetal loss rate of 10%.[59] Canlorbe et al., Poissonnier et al. and Zwiers et al. found similar results.[13,60,61]

Another downside to IUTs is the formation of additional alloantibodies, with studies reporting rates as high as 22-26% of pregnancies affected by this.[62,63] It is therefore suggested to provide extended phenotype-matched blood, including matching for Rh, K, Fy, Jk and Ss antigens of the mother if possible to prevent further antibody formation. Though this should not delay treatment.[62,63]

Intraperitoneal approach

To tackle the problems of early intravascular transfusions but also to benefit from possible prolonged interval time between procedures, the intraperitoneal approach is suggested as beneficial.[64-66] Both Nicolini et al. and Moise et al. proposed the combined approach, finding more stable hematocrit levels allowing for more time until the next procedure and less procedures overall.[64,66] Some centers adopted this combined approach, including our own center, though no reports on this are published to date.

With regards to bridging early transfusions, in the UK a ten-year case series was published on patients with severe early onset HDFN treated with intraperitoneal transfusion until the intravascular approach was possible, starting as early as 15 weeks gestation. They state intraperitoneal transfusions are technically easier causing less complications and the early transfusions before the fetus is severely affected ensure better coping with transfusion and better outcomes in general.[65] Difficulty with this approach is not knowing if fetal anemia was actually present due to the lack of fetal sampling, that is performed before intravascular transfusions but cannot be done with the intraperitoneal approach.[59]

Postponing intrauterine transfusions – noninvasive options

Because of the increased risk associated with early IUTs plasmapheresis, intravenous immunoglobulins (IVIG), immunosuppressants or combined therapy have been studied and reported to postpone the first IUT.

Therapeutic plasma exchange

Therapeutic plasma exchange (TPE) is a technique in which (maternal) plasma is removed from the circulation and is replaced with saline, plasma protein fraction or albumin.[67,68] This invasive procedure removes maternal alloantibodies and is thought to thereby prevent fatal hemolysis and anemia in immunized pregnancies. TPE was first developed for this cause in the early 1970s. Angela et al.[67] described a series of 14 high risk cases of anti-D alloimmunized pregnancies treated with TPE showing a 75% perinatal survival rate.[67] The therapy soon demonstrated to not have a long lasting effect.[20,69] Furthermore, evidence for TPE is entirely based on case-reports.[70,71] Since IUT and ultrasound techniques improved, TPE as a therapy by itself became subordinate.[20] It is, however, described to be of benefit in combination with IVIG treatment for severe (early) cases of HDFN.[69]

Combined TPE and IVIG

The largest study on this combined approach from Ruma et al.[69] describes nine patients with a history of severe HDFN.[69] They received three days of TPE, after the 12th week of gestation, followed by IVIG for two days in a loading dose. Thereafter, IVIG was continued weekly in a lower dose up until the 20th week of gestation. IUTs were required in all treated pregnancies, as expected but all nine fetuses survived, whereas in previous pregnancies seven women experienced intrauterine demise. The IUTs were performed an average 2,5 weeks later than in the previous pregnancy. The authors conclude that combined therapy is a useful option for women with a history of severe HDFN.[69]

IVIG

IVIG was long thought to have great beneficial effects in women with a history of severe HDFN. The working mechanism has been attributed to multiple factors including inhibition of the transplacental antibody transport and negative feedback on antibody levels.[58] Most evidence was limited to single center studies.[72-74] An international multicenter retrospective cohort study compared IVIG-treated pregnancies compared to non-treated pregnancies. A delay of 15 days was found for the need for IUT when compared to the non-treated group. If IVIG was started before 13 weeks of gestation a difference of 25 days was found for the onset of severe anemia in need of IUT. The overall survival was 88% and did not differ between treatment groups. Other beneficial factors that could be attributed to IVIG therapy was a lower rate of fetal hydrops and a decreased need for neonatal exchange transfusions, both associated with adverse outcome.[75]

Immunosuppression

Several immunosuppressants have been considered and explored to treat HDFN. Amongst these are promethazine, prednisolone, and azathioprine. None of these showed sufficient evidence for beneficial effects.[76] Gusdon reported in 1982 a decrease in perinatal mortality and exchange transfusions in a case series of 72 patients treated with promethazine hydrochloride, though this was not confirmed by others.[76,77] The effect of promethazine on the fetus is unknown, but could potentially increase the risk of graft-versus-host disease in fetus in need of IUTs. Therefore promethazine is not recommended in alloimmunized pregnancies.[77]

In Figure 2 we have summarized the main laboratory diagnostics, clinical diagnostics and antenatal treatment options with their advantages and disadvantages.

Perinatal and postnatal management

Perinatal management

Optimal perinatal management includes decisions on the timing of delivery and administration of antenatal corticosteroids. Both should be discussed with parents.

Timing of delivery

The best timing of delivery is in a delicate balance between allowing sufficient time for maturation of pulmonary and hepatic enzyme systems in utero versus the risks associated with continued monitoring and IUTs.

		Advantages	Disadvantages
Laboratory diagnostics	*Titers*	Highly adopted worldwide Simple techniques	Low specificity
	Bioassays	Increases predictive value compared to titers alone	Limited adopted due to complex techniques or limited added value
	Fetal antigen typing PCR	Non-invasive High sensitivity and specificity	Requires high level laboratory knowledge and technical infrastructure
Clinical diagnostics	*PSV-MCA Doppler*	Non-invasive Very low false negative rate	Highly skilled sonography required False positive rate
	Fetal blood sampling	Accurate Hb evaluation	Invasive method associated with risks
Antenatal therapies	*Immunosuppressants*	Effect not proven	Effect not proven Possible harmful effects Use in alloimmunized pregnancies not recommended
	IVIG	Early started therapy delays onset need for IUTs	High costs Adverse maternal side effects
	Therapeutic plasma exchange	Early started therapy may delay onset need for IUTs	Evidence based on limited case series
	Intrauterine transfusions	Proven therapy	Reguires experienced staff and high quality equipment
	Preterm delivery	May reduce antibody load in neonate	Preterm delivery gives neonatal related possible severe risks

Figure 2. Advantages and disadvantages of different laboratory diagnostics, clinical diagnostics and antenatal treatment options. IVIG: intravenous immunoglobulins; IUT: intrauterine transfusion.

Historically when only fetal peritoneal transfusions were performed, fetuses were delivered at 32 weeks. With improving techniques and better outcome most centers nowadays suggest a final IUT up to 35 weeks gestation and delivery at approximately 37 weeks.[7,17,78] Yet, studies on the optimal timing of delivery are limited. A study from Klumper et al. showed in a large cohort that survival rates were increased in cases treated with IUTs at or passed 32 weeks of gestation in comparison to cases receiving IUTs only up to 32 weeks of gestation.[79] However, techniques and guidelines have changed and it is hard to compare those results with today's cases. The ultimate decision for the timing of induction of labor lies with the treating obstetrician and should be closely discussed with the neonatologist and should also involve the pregnant woman and her partner. Timely counseling on what to expect on the timing of delivery and the postnatal management is an important part of expectation management in the treatment of HDFN. Importantly, the bilirubin thresholds for exchange transfusion and phototherapy are lower when delivery occurs before 37 weeks.[80] Therefore, birth at or after 37 weeks not only reduces the risks and complications due to prematurity, but will also automatically reduce the need for exchange transfusions as higher thresholds for exchange transfusions and phototherapy can be used.

Antenatal corticosteroids

Infants suffering from HDFN are often born preterm, at a gestational age below 37 weeks.[49] When preterm birth is expected, a single course of antenatal corticosteroids may be administered to accelerate fetal lung maturation and by that reducing respiratory problems.[81] This should also be envisaged in pregnancies affected by HDFN when preterm birth is imminent and pregnancy cannot be prolonged. Between 34 and 37 weeks of gestation, there is some discussion regarding administration of corticosteroids and possible negative side effects. Interestingly, several studies have revealed that antenatal betamethasone may also reduce the risk of neonatal jaundice reducing the need for phototherapy, possibly because of acceleration of liver maturation.[82,83]

Postnatal management

The two major problems arising in the newborn affected by HDFN are hyperbilirubinemia in the first few weeks of life, and (prolonged) anemia up to the first three months of life. Proper postnatal management and up-to-date knowledge on these two factors are important to reduce the risks of short and long-term morbidity and mortality.

Hyperbilirubinemia

In utero, excess fetal bilirubin resulting from hemolysis is transported via the placenta into the maternal circulation and is then processed and excreted by the mother. Whether all of

the excess fetal bilirubin is transported to the maternal circulation is unknown. Increased bilirubin in utero has been reported and can potentially also lead to neurotoxic damage in the fetus.[84] When the fetus is born, the connection to the maternal system is separated and the neonatal system has to discard the bilirubin by itself. Both the immaturity of the neonatal liver as well as the prolonged hemolysis caused by circulating maternal antibodies contribute to severe hyperbilirubinemia. When left untreated unconjugated bilirubin can cross the blood-brain barrier where it can subside in the basal ganglia, causing acute bilirubin encephalopathy or 'kernicterus'.[85,86] Postnatal treatment is focused on preventing bilirubin levels from reaching toxic levels. This is primarily done by intensive phototherapy but sometimes still require exchange transfusions when levels rise above a certain threshold or signs of acute bilirubin encephalopathy occur.[80] During an exchange transfusion, 85% of neonatal blood is replaced with donor blood, removing both excess bilirubin and maternal alloantibodies in the process. Exchange transfusions are however invasive procedures through central lines and are not without risk, requiring sufficient expertise from the treating neonatal team.[86]

Anemia

Maternal alloantibodies remain in the neonatal circulation for up to six months after birth and continue to trigger hemolysis. These alloantibodies, including alloantibodies against Kell, can also suppress the erythropoiesis causing prolonged anemia or late anemia up to three months after birth.[87] Ree et al.[88] found that IUTs caused a decrease in reticulocyte count in newborns affected by D and Kell mediated HDFN, resulting in prolonged hyporegenerative anemia and an increased need of RBC transfusions.[88] Around 80% of infants suffering from HDFN need at least one top-up transfusion,[86] with IUTs and D antigen being risk factors.[87] RBC transfusions are required up to three months after birth[89,90] and therefore weekly monitoring of Hb levels and reticulocyte counts are necessary in the first few months of life.

Other supplements and treatment options for HDFN in the newborn have been extensively described by Ree et al. in this journal before.[86]

Preconception counseling

The course of treatment for HDFN can be a long and worrisome process. After delivery the parents should be made aware by their obstetric caregiver of the option for preconception counseling before becoming pregnant again. During the preconception counseling the physician should properly inform the parents about recurrence rate and expectations in a subsequent pregnancy. Factors that should be taken into account are:

type of antigen found, zygosity of the father and the course of the ultimate pregnancy. In a subsequent pregnancy parents should be referred early in pregnancy to a center of expertise in maternal-fetal medicine. When early IUT (before 24 weeks of gestation) was necessary in the previous pregnancy then IVIG should be considered.

Future perspectives

Since its discovery, management and treatment has come a long way having a huge impact on morbidity and mortality. Future developments should focus on finding and developing better diagnostic parameters, noninvasive treatment options and optimizing treatment for fetus and newborn.

IgG Fc-glycosylation

As mentioned in chapter 2, multiple assays have been developed to estimate disease severity. Those tests can be used for high risk selection and to guide perinatal management. An interesting current field of research is to explore if determination of the sugar-moiety attached to the Fc-tail of RBC alloantibodies correlates with the pathogenicity of those antibodies. Through binding of the IgG alloantibodies to the fetal RBCs, the Fc-tail can be bound by phagocytes of the reticulo-endothelial system in the spleen. Phagocytes carry different types of Fc receptors, namely Fc-RIa, F-RIIa and FcRIIIa. The binding region of the Fc domain has a sugar moiety (glycan) attached to it.[91] The composition of this glycan determines the affinity of the IgG molecule to the IgG Fc receptor type IIIa.[22] In 2014 Kapur et al. found lowered Fc core-fucose in anti-D specific IgG1 to be associated with increased ADCC levels and decreased fetal or neonatal hemoglobin levels.[22] Similar results were found in a different study from this research group when looking at fetal or neonatal alloimmune thrombocytopenia (FNAIT).[92] These results suggest that the IgG-Fc-glycosylation is important in the destructive capacity of antibodies and could be a potential biomarker for HDFN. These results were supported in a prospective study published in 2017 in which researchers found that altered galactosylation and low fucosylation are associated with developing HDFN.[93] It would be interesting to evaluate the predictive value of IgG-Fc-glycosylation for the occurrence of fetal anemia and the need for IUTs in larger sample size.

Neonatal Fc Receptor blockers

In chapter 3 we discussed antenatal therapeutic options for severe HDFN including their downsides. The main challenge is to find a treatment option that is non-invasive. Biological agents targeting the Fc receptor, also present on the placenta, try to overcome this challenge. By attaching to the receptor with high affinity they block the binding of

pathogenic IgG molecules prohibiting the transfer to the fetus. What is more, the Fc receptor is responsible for maintaining a long half-life of IgG molecules. By blocking the receptor the IgG levels in maternal plasma decreases. Though promising, potential risks that can occur include increased risk of infection in both mother and neonate due to the reduction of circulating IgG molecules. Postpartum the neonate might need IVIG supplementation for its hypogammaglobulinemia. What is more, maternal albumin levels also decline due to intracellular recycling, increasing the risk of (severe) edema formation.[94,95] Several of these biological agents are being tested in adults for various IgG mediated immune diseases. One of them is evaluated in pregnant women in a phase 2 trial to determine the efficacy for treatment in HDFN. This monoclonal antibody Nipocalimab (M281) is being evaluated in a multi-center open label-study pregnant where alloimmunized women with high risk of severe HDFN are included (NCT03842189). This study may serve as a proof of concept. In phase 1 trials Nipocalimab showed to reduce circulating IgG levels up to 85%.[95] If these studies show that these blockers can effectively block transfer of pathogenic IgG, this therapy not only can reduce the development of HDFN but also other IgG mediated immune diseases such as FNAIT.[87,94]

Umbilical cord blood for intrauterine transfusions

Umbilical cord blood (UCB) collection and transfusion is an interesting field of research which aims to improve RBC transfusion for (preterm) neonates. RBC transfusions with adult donor blood are suggested to be linked to multiple complications such as necrotizing enterocolitis, bronchopulmonary dysplasia and retinopathy of prematurity.[96] These complications are attributed to the fact that adult whole blood contains HbA as opposed to fetal blood which contains predominantly HbF. HbF is known to have a higher affinity for oxygen, necessary in the more hypoxic environment in utero[97] and may therefore have a protective role from oxidative stress. Recent small studies have suggested that transfusions with UCB with predominantly HbF would be more physiological and could decreased the morbidity rate associated with RBC transfusions.[98,99] More research is needed to determine if UCB is indeed preferable to adult blood for fetuses and preterm neonates. In addition, extensive collaboration between cord blood banks should be established and issues involving storage and typing need to be addressed.[97]

Erythropoietin

Erythropoietin (EPO) is necessary to stimulate erythropoiesis. Plasma levels in neonates are lower than in children and adults and this is one of the causes of anemia in (preterm) neonates. Stimulating erythropoiesis by administering EPO has therefore been studied

to treat neonatal anemia with varying results.[100,101] (Repeated) IUTs have also been shown to be a strong contributing factor in the suppression of compensatory erythropoiesis.[88] A randomized controlled trial has therefore been performed in our center to assess the effect of exogenous administered darbepoetin after birth on postnatal transfusion dependency in IUT treated infants (NCT03104426). First results are expected by the end of 2022.

Conclusion

With adequate early identification of pregnancies 'at-risk', efficient prevention programs and timely referral to a center of expertise in maternal-fetal medicine, fetal hydrops, intrauterine demise and long-term morbidity can be reduced or even avoided. Future technologies focus on improving diagnostic tools to be more specific and universally applicable and developing (noninvasive) treatment options that will help reduce mortality and morbidity even further.

Expert opinion

In the last decades HDFN has gone from being one of the main reasons for perinatal death to being near eradicated in high-income countries. A lot of scientific knowledge of HDFN originated from Europe and the USA, with a high percentage of Caucasian residents. We know that in Caucasians approximately 15% of women is RhD-negative. In Black women this percentage lies between 2-5% whereas in Japan, China and South East Asia women are rarely RhD-negative.[102,103] Though numbers for most countries depend on estimations due to the absence of national registries.[104] Improved recording of RhD-negative frequencies and the causes of HDFN will improve management in countries other than Europe and the USA. For instance, in the Asian population, HDFN due to anti-K is very uncommon, yet anti-M has a high incidence and pathogenicity. In the Caucasian population around 0.1% has anti-M of the IgG class, in the Asian population this is 57.4% ~ 84.0%, explaining why it is a much more common cause of HDFN.[26] It is highly valuable to get a better view of the differences in genetic occurrence of antigens but also genetic variants of (D) antigens, which also has an effect on the immunogenicity. Even though there is no prophylaxis for these antigens, the awareness of these differences early in pregnancy and adequate risk analysis based on these differences will allow for early and adequate treatment. Therefore, understanding these differences will help decipher the pathogenesis of HDFN and interaction between antigens, antibodies and the Fc receptor even further.

In high-income countries morbidity and mortality is rare and the time is now to ensure global reduction in prevalence of HDFN.[105] Worldwide anti-D mediated HDFN is estimated to still account for 160.000 perinatal deaths each year and 100.000 cases of disability.[104] Much of these perinatal deaths could have been avoided with proper identification and prophylaxis. This burden is especially high in South Asia and Sub-Saharan Africa.[104] Since the introduction of RhIg in 1968 there has been a reduction of approximately 50% of anti-D mediated HDFN. The FIGO Committee for Safe Motherhood and Newborn Health recently published an article addressing this problem and came up with recommendations to improve prophylaxis.[106] Creating awareness for this problem on the global health agenda and a systematic national approach in each country seem to be key building blocks for an effective prevention strategy.[105] We are faced with the challenge to establish improved care in an affordable manner. A challenge in which high-income countries could collaborate with medium- and low-income countries in a shared effort to implement step-by-step evidence-based prevention, screening and disease treatment measures. Highly technical and costly procedures such as IUTs might not be feasible yet, though the biggest gain in countries without an effective prevention program is to deploy effective prophylaxis programs. In addition, timely onset of intensive phototherapy will help reduce the morbidity even further. If sustainable programs are implemented this will add to systemically resolve preventable perinatal deaths and a reduction in morbidities.

Highlights/Key issues

- HDFN still causes severe morbidity and mortality in the fetus and newborn.
- Laboratory management should focus on optimal prevention of D alloimmunization in D-negative women and timely administration of RhIg, with additional doses in cases of suspected FMH.
- Timely referral to a center of expertise in maternal-fetal medicine and IUT treatment significantly improve fetal and neonatal outcome.
- Postpartum care mainly focusses on hyperbilirubinemia and (late) anemia. Adequate treatment of both reduces (long term) morbidity.
- Future technologies focus on improving diagnostic tools to be more specific and universally applicable and developing (non-invasive) treatment options that will help reduce mortality and morbidity even further.
- Worldwide, anti-D mediated HDFN still accounts for 160.000 perinatal deaths and 100.00 patients with disabilities every year. By implementing sustainable prevention, screening and disease treatment measures in all countries this will systemically reduce unnecessary perinatal deaths.

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