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Hemolytic disease of the fetus and newborn: awareness precedes change

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CHAPTER
1

History And Current Standard Of Postnatal Management In Hemolytic Disease Of The Fetus And Newborn

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ABSTRACT

Since the discovery of the Rh blood group system in 1940, a greater understanding of hemolytic disease of the fetus and newborn (HDFN) was gained. In the years thereafter, researchers and clinicians came to the current understanding that fetal and neonatal red blood cells (RBC) are hemolyzed by maternal alloantibodies directed against RBC antigens potentially leading to severe disease. Preventative measures, such as Rhesus(D) immunoprophylaxis (RhIG), have greatly decreased the prevalence of Rh(D)-mediated HDFN, although a gap between high-income countries and middle- to low-income countries was created largely due to a lack in availability and high costs of RhIG. Other important developments in the past decades have improved the identification, monitoring, and care of pregnancies, fetuses, and neonates with HDFN. Prenatally, fetal anemia may occur and intrauterine transfusions may be needed. Postnatally, pediatricians should be aware of the (antenatally determined) risk of hemolysis in RBC alloimmunization and should provide treatment for hyperbilirubinemia in the early phase and monitor for anemia in the late phase of the disease. Through this review, we aim to provide an overview of important historic events and to provide hands-on guidelines for the delivery and postnatal management of neonates with HDFN. Secondarily, we aim to describe recent scientific findings and evidence gaps.

Conclusion: Multiple developments have improved the identification, monitoring, and care of pregnancies and neonates with HDFN throughout the centuries. Pediatricians should be aware of the (antenatally determined) risk of hemolysis in RBC alloimmunization and should provide treatment for hyperbilirubinemia in the early phase and monitor for late anemia in the late phase of the disease. Future studies should be set in an international setting and ultimately aim to eradicate HDFN on a global scale.

WHAT IS KNOWN:

- Developments have led to a greater understanding of the pathophysiology, an improved serological identification and monitoring of at-risk cases and the current pre- and postnatal treatment.

WHAT IS NEW:

- This review provides the pediatrician with hands-on guidelines for the delivery and postnatal management of neonates with HDFN.
- Future studies should be set in an international setting with the ultimate aim of eradicating HDFN.

INTRODUCTION

In hemolytic disease of the fetus and newborn (HDFN), fetal and neonatal red blood cells (RBC) are hemolyzed by maternal alloantibodies directed against RBC antigens. Antenatally, hemolysis may lead to fetal anemia, and treatment with intrauterine transfusions (IUT) may be necessary. Postnatally, continued hemolysis may lead to severe hyperbilirubinemia in the first 1–2 weeks which may be treated with intensive phototherapy and, if needed, exchange transfusions. Lastly, anemia may occur until three months postnatally due to continued hemolysis or an inhibition of erythropoiesis, and RBC transfusions may be needed.

Many major historic events in the field of HDFN have occurred and led to a greater understanding of the pathophysiology, an improved identification and monitoring of at-risk cases and the current treatment of fetuses and neonates with HDFN. Through this review, we aim to provide an overview of important historic events and to provide hands-on guidelines for the delivery and postnatal management of neonates with HDFN. Secondarily, we aim to describe recent scientific findings and evidence gaps.

HISTORIC HIGHLIGHTS

In 400 BC, Hippocrates, considered to be the founder of modern medicine, first described a fetus carnosus or “fleshy fetus,” which is now believed to have been hydrops fetalis (Figure 1). It was not until 1609 that a French midwife Louise Bourgeois reported on a twin with hydrops fetalis, severe jaundice, and kernicterus.[1] Dozens of similar reports were made in the centuries thereafter, reporting on “universal edema of the fetus,” “fetal leukemia,” “unusual blood-forming foci in the liver,” and a “familial incidence.” In 1932, a review was published in which these clinical entities were first linked together, but the underlying pathophysiology remained unknown.[2] It was not until the efforts of Landsteiner starting with identification of ABO blood group system in 1900[3], followed by Rh in the 1940s that the pathophysiology of the disease was uncovered.[4] A year later, Levine and Stetson published on pregnancy-related alloimmunization.[5] Thereafter, many developments in the prevention of alloimmunization through Rh(D) immunoprophylaxis (RhIG)[6-9] and K (also known as Kell)-negative blood transfusions in women of childbearing age[10], antenatal serological identification and monitoring[11], and antenatal[12-16] and postnatal treatment options were made. Cumulatively, these developments led to a great decrease in the prevalence and severity of the disease, through timely identification and prompt treatment.

Several advances in the treatment of hyperbilirubinemia were made, including exchange transfusions and phototherapy. Exchange transfusions were first performed

by Wallerstein in 1946, who described a procedure to remove “a factor of toxemia” by simultaneous removal of Rh-positive blood and administration of Rh-negative blood.[17] This successful but risky procedure became the cornerstone of neonatal hyperbilirubinemia management. In vitro photosensitivity of bilirubin was first described in 1957.[18] British nurse Jean Ward was the first to notice a decrease in neonatal jaundice upon sunlight exposure. This was further investigated by Cremer, Perryman, and Richards leading to the creation of an “artificial-light treatment” in 1958.[19] At that time, the authors concluded that “no prospect can be entertained that this light treatment will prove a substitute for exchange transfusion in the erythroblastotic infant with active haemolysis…” South-American and European countries embraced phototherapy in the next decade[20], whereas skepticism prevailed in North America. Phototherapy was accepted as the first-line treatment for neonatal hyperbilirubinemia many years after publication of a randomized controlled trial by Jerold Lucey in 1968 that proved the efficacy of phototherapy for neonatal hyperbilirubinemia.[21,22]

PATHOPHYSIOLOGY AND PREVENTION OF ALLOIMMUNIZATION

HDFN is caused by maternal alloantibodies directed against the child’s erythrocytes. Maternal alloimmunization may occur in incompatible blood transfusions or through fetomaternal hemorrhage in pregnancy. During pregnancy, IgG is actively transported across the placenta, and in the fetus, monocyte-mediated hemolysis may lead to fetal anemia if the compensatory erythropoiesis is insufficient. Postnatally, hemolysis could lead to (severe) hyperbilirubinemia in the first one to two weeks after birth and anemia in the first three months of life. Not all alloantibodies are clinically relevant. Anti-Rh(D), anti-K (anti-K1), and anti-Rh(c) are strongly associated with severe HDFN. Anti-Rh(E) sometimes results in severe HDFN, but mostly mild. Severe HDFN is rarely caused by other Rh-antibodies and non-Rh antibodies.[11] Anti-K and in rare occasions anti-M and anti-Ge are known to suppress erythropoiesis that may consequently cause anemia.[23-27]

In general, anti-A and anti-B do not lead to fetal anemia as these naturally occurring antibodies are mostly of the IgM class and therefore do not cross the placenta. However, in some cases, a mother produces clinically relevant titers of anti-A or anti-B of IgG class, which will be transported to the child. Taken together with the fact that A and B antigens have a lower expression on fetal and newborn erythrocytes and are expressed on a wide variety of human tissues that may influence the extravascular absorption of IgG, this also contributes to a generally mild disease without fetal anemia. Postnatally, however, ABO-antagonism may invoke serious hyperbilirubinemia that may require intensive phototherapy and exchange transfusions. Since routine screening

programs are not aimed to detect anti-A or anti-B, high levels of bilirubin in ABO-antagonism may be detected late. Triggers to produce high IgG titers anti-A or anti-B are not known yet and may differ in various parts of the world.[28-31]

Figure 1: Major events in the understanding and management of hemolytic disease of the fetus and newborn. Rh, also known as Rhesus; RCT, randomized controlled trial; HDFN, hemolytic disease of the fetus and newborn; IUT, intrauterine transfusion.

PRENATAL IDENTIFICATION, MONITORING, AND CARE

In most countries, a pregnant woman is screened for the presence of erythrocyte alloantibodies early in pregnancy, with a repeated screening later in pregnancy for all or for a subset of women. In this way, interventions can be initiated to prevent severe disease and even the death of the child.[11] Recent developments in the serological identification and prenatal monitoring are discussed in detail elsewhere.[32] If erythrocyte alloantibodies are found, it is important to know if the child is positive for the implicated erythrocyte blood group antigen. An optimal method to determine the fetal antigen status is noninvasive fetal blood group typing with cell-free fetal DNA (cffDNA) from maternal plasma. But, in settings in which cffDNA measurements are not possible, fetal testing through invasive amniocentesis may be performed. Cases at risk of severe or mild HDFN are mostly identified through sequential alloantibody titers, using indirect antiglobulin tests performed in saline, until a critical titer is found. The titer is the dilution of maternal serum in which a positive indirect antiglobulin test is still found, reported as 1:1, 1:2, 1:4, and so forth. The higher the denominator, the higher the titer. Several cutoff values are determined per alloantibody type. Additionally, biological assays to determine the hemolytic activity of alloantibodies, such as the antibody-dependent cellular-cytotoxicity (ADCC) assay, may be employed to increase the positive predictive value.[33,34] Its use in practice is limited due to its complexity, costs and use of radioactive material. Currently, the ADCC is solely used in the Netherlands.

The presence and severity of fetal anemia can be assessed by quantifying the bilirubin levels in amniotic fluid through invasive amniocentesis. In recent years, Doppler sonography allowed for noninvasive detection of early fetal anemia by assessing the peak systolic velocity of the middle cerebral artery (PSV-MCA). A PSV-MCA of more than 1.5 multiples of the median was found to have a higher diagnostic accuracy for severe fetal anemia in comparison to determination of bilirubin in amniotic fluid[35] and is therefore predominantly used in practice. It is essential to timely treat fetal anemia with IUT to prevent hydrops fetalis, a consequential accumulation of fluids in the extravascular space, as hydrops fetalis is associated with adverse perinatal outcome and long-term neurodevelopmental impairment.[36]

DELIVERY

If one or more IUTs were given, or if the risk of hemolysis is regarded as high (due to high antibody titers or high results in biological assays), then induction of labor or a planned caesarean section is generally advised to occur at a gestational age of 36–37 weeks. In the last four weeks of gestation, the active fetomaternal transport of IgG significantly increases and thereby risks of severe hemolysis.[37] Risk of preterm

birth and risks inherent to IUTs should be considered when signs of fetal anemia are found at 34–35 weeks. In practice, significant differences in gestational age at birth may exist, due to (un)availability of prenatal treatment options. Some centers may be inclined to induce delivery at 32–34 weeks, potentially leading to more neonatal morbidity, although there is a general lack of evidence on the impact of preterm and term delivery of fetuses with severe HDFN.

Garabedian et al. assessed the effect of delayed cord clamping (DCC), in neonates treated with ≥1 IUT, through a retrospective before/after study. DCC was defined as cord clamping 30 seconds after birth. They found that the need for exchange transfusions was lower in the group with DCC and that DCC led to a delay of the first RBC transfusion for postnatal late-onset anemia[38] suggesting that DCC is beneficial for the postnatal management. However, no randomized controlled trials for DCC in HDFN exist, and theoretically, DCC may lead to an increased load of antigen-positive erythrocytes and maternal IgG.

At delivery, the hemoglobin level (Hb) and reticulocyte and platelet count should be determined in cord blood to promptly identify anemia and potentially associated thrombocytopenia, discussed below.[39] Additionally, the total serum bilirubin (TSB) and albumin level should be determined.

POSTNATAL MONITORING AND CARE

Early phase: hyperbilirubinemia

Monitoring and treatment in the early phase (1–2 weeks after birth) focus on the early recognition and prevention of complications of severe hyperbilirubinemia. Postnatal monitoring and treatment are also described in Figure 2. Phototherapy is the ubiquitous first-line treatment for hyperbilirubinemia and prevention of exchange transfusions, due to its effectiveness and minimal risk of adverse events with correct application. Phototherapy causes configurational and structural isomerization and photooxidation of bilirubin. The water-soluble bilirubin isomers can be excreted without hepatic conjugation in bile (and urine) thereby lowering TSB. Fluid supplementation, whether intravenously or enteral, may enhance TSB reduction, but evidence of beneficial effects on long-term neurological outcome is lacking.[40] Effectiveness of phototherapy, however, largely relies on the intensity of irradiance, distance between the light and neonate, and exposed body surface area. The American Academy of Pediatrics (AAP) guideline on the management of hyperbilirubinemia states that intensive phototherapy should be applied to as much body surface area as possible using a narrow-spectrum

light-emitting diode (LED) blue light with an irradiance of 30 μW/cm2 per nm at a wavelength between 460 and 490 nm.[41] Narrow-spectrum LED devices may be more effective in lowering TSB in comparison to non-LED devices (i.e., fluorescent lamps or halogen lights); LED devices produce high irradiance intensity and less heat and may thus safely be placed closer to the neonate. A systematic review and meta-analysis evaluated the effectiveness of LED devices in comparison to non-LED phototherapy in lowering TSB. Data from four randomized controlled trials including infants with a gestational age of ≥35 weeks were assessed, and the authors found no significant differences in the rate of TSB decrease between LED and non-LED phototherapy devices.[42-45] The TSB-lowering effect of LED devices might have been diminished by a smaller body surface area exposed to LED light, thereby decreasing the spectral power (light irradiance x surface area).[46] In addition, the studies included in the systematic review utilized different types of LED. The authors therefore conclude that future studies should evaluate the effectiveness of different LED devices, report on the exact exposed body surface area, and use irradiance mapping techniques.[46] In clinical practice, double-sided LED phototherapy with an intensity of at least 30 μW/cm2 per nm is recommended for intensive phototherapy. LED phototherapy is found to be superior to non-LED phototherapy due to the combination of its efficacy and a long life of the LED light source.

Phototherapy is generally regarded as a safe, noninvasive treatment to prevent the need for invasive exchange transfusions. Some studies suggest an association between phototherapy and autism[47], cancer[48,49], and diabetes[50] although these studies were unable to correct for TSB levels. Newman et al. found a small increased risk of childhood seizures, defined as a seizure diagnosis with ≥1 antiepileptic drug prescription, after correcting for TSB levels.[51] Das and Naik reported increased odds for the occurrence of asthma and allergic rhinitis, although the level of evidence was low.[52] In preterm infants, LED phototherapy at irradiances up to 35 μW/cm2/nm did not affect levels of an oxidative marker of DNA damage.[53]

Neonates with a prenatally identified risk of severe hyperbilirubinemia, due to high maternal alloantibody levels, high ADCC results, or previous treatment with IUT, should receive phototherapy within the first hour after birth, or as soon as possible, after delivery. Skin-to-skin contact, in case of a good transition after birth, however, should be taken into account. Severe hyperbilirubinemia may be expected in Rh(D) and Rh(c) alloimmunization, whereas anti-Rh(E), anti-Rh(e), and anti-Rh(C) generally lead to a more mild disease.[11] Intensive phototherapy may be started later in infants born to mothers with a low alloantibody level and immunization to Rh(E), Rh(e), and Rh(C), based on TSB levels and TSB trajectory. K-alloimmunization is associated with an

inhibition or destruction of RBC progenitors and less with hemolysis of mature RBCs. Thereby, neonates with K-mediated HDFN experience less and lower levels of bilirubin. In a retrospective study performed at our center, comparing K-mediated HDFN to Rh(D)-mediated HDFN in a cohort between 2000 and 2008, neonates with K-mediated HDFN were found to require less phototherapy (2.4 versus 4.1 days) and less exchange transfusions (6 versus 62%).[54] Between 2009 and 2021, no exchange transfusions were performed in neonates with K-mediated HDFN at our center (n = 0/43, non-published organizational data).

TSB in neonates with HDFN receiving phototherapy should be measured immediately at birth and then every 3-4 hours, but the frequency may be increased depending on the gestational age, risk factors, TSB levels, and trajectory. Additionally, measurement of end-tidal carbon monoxide concentration, corrected for ambient carbon monoxide (ETCOc), may be used as a potentially useful method for quantifying the rate of hemolysis. During heme catabolism, carbon monoxide is produced in equimolar amounts with bilirubin. Therefore, this noninvasive method may provide a more accurate way to identify infants at risk for developing severe hyperbilirubinemia in comparison to the antiglobulin test.[41,55-58] Pediatricians ought to be aware of potential neurotoxicity risk factors that may exist besides HDFN and may lead to an increased susceptibility for bilirubin encephalopathy. These risk factors include a gestational age <38 weeks, isoimmune hemolytic disease (i.e., positive direct antiglobulin test), glucose-6-phosphate dehydrogenase deficiency, or other hemolytic conditions, sepsis, clinical instability within the last 24 hours and albumin <3.0 g/dL.[41]

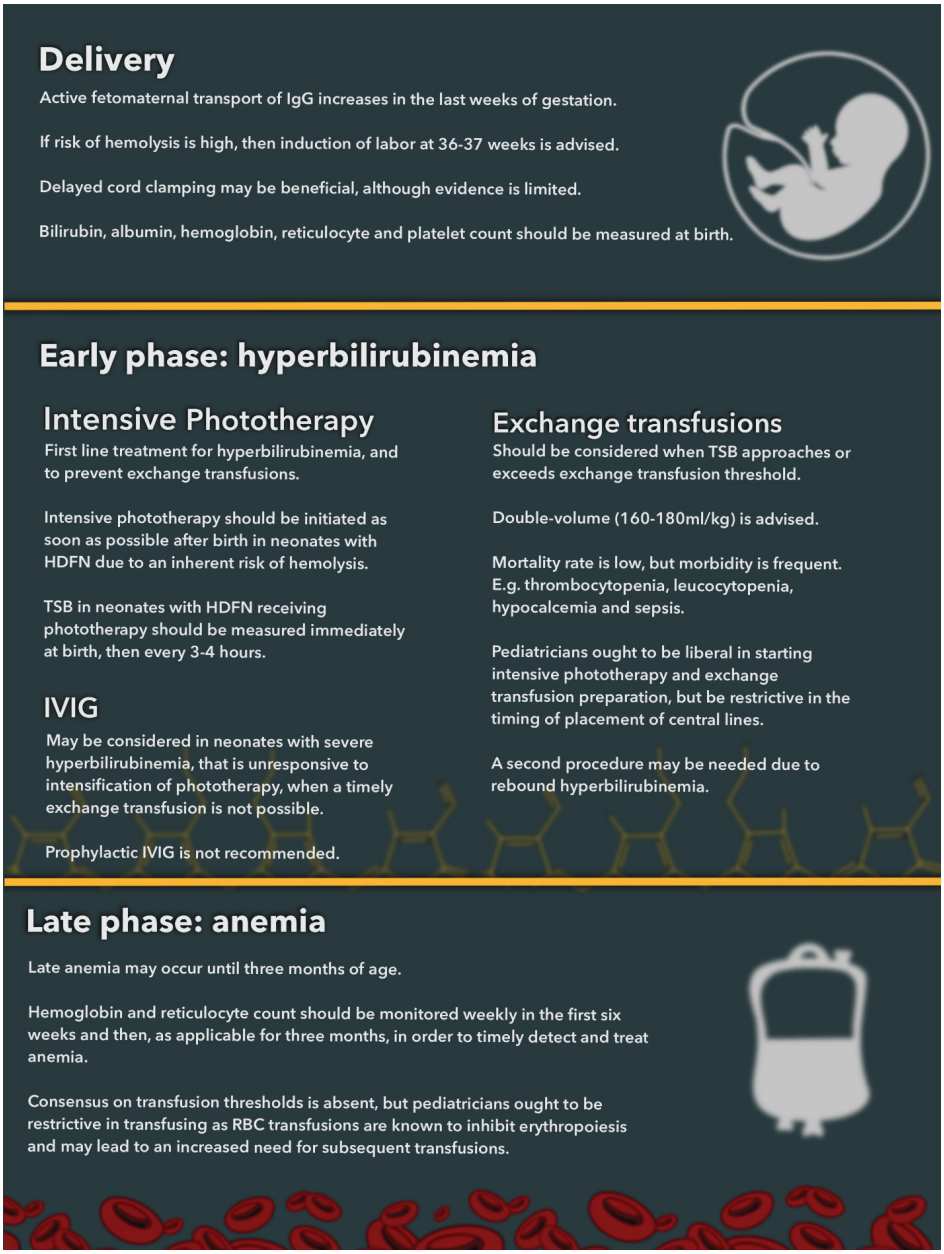

Figure 2: Advise for the delivery and postnatal monitoring and treatment of HDFN. IgG, immunoglobulin G; IVIG, intravenous immunoglobulins; HDFN, hemolytic disease of the fetus and newborn; TSB, total serum bilirubin; RBC, red blood cell

In vivo, albumin binds and transports unconjugated bilirubin to the hepatocytes surface and thereby facilitates the hepatic conjugation of bilirubin. In case of hypoalbuminemia or bilirubin displacing drugs, a relatively greater amount of unbound bilirubin coincides with an increased risk of bilirubin neurotoxicity. Measurement of unbound bilirubin, which is more closely correlated to neurotoxicity than TSB[59-63], is in clinical practice rarely performed due to the inability of most laboratories to routinely measure unbound bilirubin concentrations. Currently, jaundice management guidelines with treatment thresholds that are also based on levels of unbound bilirubin only exist in Japan[64] and a reference hospital in France.[65,66] The bilirubin-albumin (B/A) ratio, in addition to TSB levels, may be used in the management of hyperbilirubinemia and should be calculated in case of impending ET.[41] A randomized controlled trial, however, found no differences in neurodevelopmental outcomes in preterm infants (GA < 32 weeks) managed with B/A ratios and TSB levels in comparison to TSB levels only, possibly due to lack of contrast between the two treatment groups.[67]

Transcutaneous bilirubin (TcB) measurements, a noninvasive method to estimate TSB levels, may be employed in all newborn infants to screen for severe hyperbilirubinemia. TcB screening has been shown to reduce blood draws and—when universally applied—reduce the incidence of hyperbilirubinemia[68-73], but a degree of uncertainty should be taken into consideration, and importantly, its accuracy in non-ABO HDFN has not been assessed. In addition, even though TcB values correlate to unbound bilirubin levels, unbound bilirubin only represents approximately 50% of bilirubin deposited in skin.[74]

When TSB approaches or exceeds critical values, defined in the newly published AAP revised guidelines in 2022, exchange transfusions may be considered.[41] In exchange transfusions, bilirubin and maternal IgG are partially removed from the neonatal circulation, and neonatal blood is replaced with donor blood, if available irradiated, leucocyte-depleted, antigen-negative blood to prevent further hemolysis and a subsequent TSB increase. Double-volume exchange transfusions (160–180 mL/kg) are standard of care as the advantages or disadvantages of single-volume exchange transfusions could not be assessed in previous studies.[75,76] Pediatricians should be aware of potential post-exchange TSB increase, i.e., rebound hyperbilirubinemia, due to a new extra- and intravascular equilibrium. In 2012, a randomized controlled trial was performed to evaluate the efficacy of a two-stage single-volume technique (TSSV) in decreasing post-exchange serum bilirubin increase in comparison to single-stage double volume. A TSSV led to significant reductions in rebound hyperbilirubinemia, and the need for repeat exchange transfusions was lower.[77] Lastly, a retrospective study found no difference in TSB reduction between the “push-and-pull” technique versus isovolumetric technique in lowering TSB in 114 neonates.[78]

Exchange transfusions may be performed using a single-site central venous access (e.g., via an umbilical vein catheter), or through a double-site procedure with arterial and venous access (e.g., via an umbilical vein catheter and umbilical artery). Central venous access may also be achieved through the brachiocephalic vein and larger peripheral veins.[79,80] In rare occasions, venous access through the femoral or internal jugular vein, in case of severe dehydration and consequent collapse of peripheral veins, may be needed.[80]

Inherent to invasive nature of the procedure and the use of central lines, exchange transfusions are not without risk. Although in experienced hands the mortality rate of exchange transfusions may be as low as 0–0.3% in (near-)term neonates with increasing rates at a lower gestational age, complications associated with exchange transfusions should be considered.[80-83] Frequently occurring complications are thrombocytopenia < 100.109/L (31≥90%)[81,84], hypocalcemia (1–47%)[81,82,84], leukocytopenia < 5.109/L (33%) and sepsis related to the procedure (10%)[81]. In a study by Jansen et al., it was found that neonates with HDFN with a central line had a higher risk for late-onset sepsis (RR 1.11, 95% CI: 1.04-1.20). Interestingly, in 32% of neonates with a central line for impending exchange transfusion, no exchange transfusion was performed, witnessing the efficacy of (intensive) phototherapy.[85] This observation is in line with the recent data from the Dutch perinatal audit on severe neonatal hyperbilirubinemia: 30 out of 109 infants with TSB levels above exchange transfusion thresholds received a double-volume exchange transfusions, next to intensive phototherapy, again witnessing the efficacy of (intensive) phototherapy.[86] Taken together, pediatricians ought to be liberal in starting intensive phototherapy and preparation of exchange transfusion in the case of severe neonatal hyperbilirubinemia and perform an exchange transfusion whenever signs of acute bilirubin encephalopathy exist but be restrictive in timing of the placement of central lines for impending exchange transfusions, to prevent an increased risk of sepsis.

Intravenous immunoglobulins (IVIG) may be used to prevent or delay severe hyperbilirubinemia and exchange transfusions, although evidence is limited.[87] In a recent systematic literature review, evidence on a single early dose prophylactic IVIG in Rh-mediated HFDN was accumulated. Seven studies reporting on neonates receiving phototherapy versus phototherapy and prophylactic IVIG were compared, and a lower need for exchange transfusions was found. But the results from these studies should be interpreted with caution due to a high risk of bias. In three of the included studies, with low risk of bias, no significant difference was found in the need for exchange transfusions in a total of 190 neonates.[88-92] Based on the available evidence, prophylactic IVIG should therefore not be routinely administered to neonates with

HDFN to prevent exchange transfusions. The role of IVIG in situations in which intensive phototherapy and/or exchange transfusions are not readily available, however, is unclear.[92] According to the recent AAP management guideline, IVIG may be considered in neonates with HDFN with a TSB concentration reaching or exceeding the exchange transfusion threshold that is unresponsive to an intensification of phototherapy if available and when a timely exchange transfusion is not possible.[41] In such situations, pediatricians ought to take into consideration both the available evidence and potential harms of proposed treatments, as well as clinical (personal) experience.

Late phase: late anemia

Maternal alloantibodies remain in neonatal circulation for three to four months and may cause so called "late anemia." Etiology of late anemia is split into late hyporegenerative anemia and late anemia of hemolytic disease, although anemia in HDFN is often a combination of the two. Hyporegenerative anemia is characterized by a low reticulocyte count, possibly due to a disruption of erythropoiesis, although the exact pathophysiology remains unknown. Late anemia of hemolytic disease is caused by continued hemolysis and is characterized by a normal or increased reticulocyte count.[93] Late anemia may be treated with RBC transfusions to (temporarily) increase the Hb level and to bridge the gap until the infants' erythropoiesis is adequate and maternal alloantibodies are cleared. Ree et al. found that 88% of neonates treated with IUT and 60% of neonates without IUT required a median of two RBC transfusion, underlining the essence of postnatal monitoring.[94] Infants ought to be monitored weekly for Hb and reticulocyte count initially for the first 6 weeks and then, as applicable, till 3 months of age. Internationally, consensus on cutoff values, dosage, and transfusion rates for neonatal RBC transfusions is absent. In the Netherlands, RBC transfusions with irradiated donor blood of 15 mL/kg at a rate of 5 mL/kg/hour are performed if the Hb falls below 10.4 g/dL (6.5 mmol/L) in the first week of life, below 8.8 g/dL (5.5 mmol/L) in the second week of life, and below 7.2 g/dL (4.5 mmol/L) from week 3 onwards. Using these guidelines, we observed that 97% (188/193) of neonates treated with IUT and 99% (104/105) of neonates not treated with IUT had a first transfusion ≤45 days after birth, suggesting that the risk of a need for a first transfusion after 45 days is low, in both groups.[94]

Treatment with exchange transfusions is associated with a postponed occurrence of late anemia, which can be explained by the replacement of antigen-positive erythrocytes with antigen-negative erythrocytes and maternal IgG removal.[54]

Comorbidities

At birth, 70% of neonates with alloimmune HDFN may have signs of iron overload (ferritin >95th percentile), decreasing to 50% at 1 month and 18% at 3 months of age. Increased ferritin levels are associated with IUTs, but also neonates that did not receive IUTs are susceptible to iron overload. Iron deficiency, on the other hand, does not occur in HDFN, and thus, iron supplementation is contraindicated.[95] Thrombocytopenia (150 *109/L) and severe thrombocytopenia (<50*109/L) occur in 26% and 6% of neonates, respectively.[39] Thrombocytopenia in HDFN is generally self-limiting, and platelet transfusions are only needed if platelet levels drop below <25 *109/L.[96] Cholestasis, or conjugated hyperbilirubinemia, occurs in 13% of neonates and, similar to thrombocytopenia, is self-limiting often requiring no further diagnostics or treatment within the first months of life.[97] Lastly, as mentioned here above, sepsis occurs in 6.2% of neonates with HDFN, particularly in those with central lines.[85]

NEURODEVELOPMENTAL OUTCOMES

Timely identification and treatment with intensive phototherapy and exchange transfusions are performed to ultimately prevent kernicterus spectrum disorder[98], a severe and permanent form of brain injury characterized by abnormal (oculo)motor function and auditory complications.[99] However, an exact definition of kernicterus and consensus on the diagnostic approach is lacking, partially due to unavailability of diagnostic methods, such as MRI, in some countries. Little data is available on the incidence, ranging from 1 to 4 in 100,000 live births, with data mostly originating from Europe and Northern America.[100]

In a cohort of 291 children at a median age of 8.2 years treated with IUTs at our center, the incidence of neurodevelopmental impairment was found to be 4.8%, slightly higher than in the general population. Importantly, hydrops fetalis was found to be strongly predictive for impaired neurodevelopment[36], underlining the importance of preventing hydrops fetalis through timely antenatal serological identification, monitoring, and treatment. Also, in a cross-sectional cohort study evaluating Dutch children treated with IUTs, it was found that the school performance and behavioral outcomes are similar to the Dutch norm and thus that favorable outcomes are to be expected.[101] Lastly, developmental outcomes of children treated with IUTs that were included in a randomized controlled trial to evaluate the effectiveness of IVIG to prevent exchange transfusions were assessed. No difference in the rate of neurodevelopmental adverse outcomes was found between the placebo and interventional group.[102]

Of note, many cohort studies on long-term neurodevelopmental outcomes in children with HDFN were performed in the Netherlands at the Leiden University Medical Center. Differences in the antenatal and postnatal management of HDFN may exist between countries and centers, due to sociodemographic and economic factors and geography that may influence the long-term outcomes. Taken together, this emphasizes the need for studies on neurodevelopmental outcomes in other centers. Additionally, little data on long-term outcomes after exchange transfusions for severe hyperbilirubinemia exists.

FUTURE PERSPECTIVES

In the past decades, important developments in the antenatal and postnatal management of HDFN have occurred (Figure 1), but several evidence gaps still exist (Figure 3). Research therefore focuses on new pharmacotherapeutic options to prevent antenatal and postnatal transfusions. An ongoing randomized controlled trial is the EPO-4-Rh study (NCT03104426) that aims to evaluate the effect of darbepoetin alfa on reducing the need for RBC transfusions, and results are expected to be available by the end of this year. Additionally, a phase 2 study to evaluate the safety and effectiveness of nipocalimab in early-onset severe HDFN is currently being carried out and might pave the way for an alternative antenatal management option to reduce the need of intrauterine transfusions (NCT03842189).

Due to the rarity of the disease, research on the treatment and clinical outcomes of HDFN is dependent on international collaboration. No studies that evaluate the international differences in management and the clinical outcomes in HDFN have yet been performed. We have therefore initiated the DIONYSUS study (worlDwIde cOllaboratioN for hemolYtic diSease of the fetUS and newborn), a multicenter international study, with the primary aim of assessing differences in prenatal and postnatal management strategies and clinical outcomes of HDFN in cases with moderate to severe HDFN due to Rh, K, or another type of RBC antigen alloimmunization, managed in expert fetal therapy centers worldwide.

Also, since the introduction of RhIG, the prevalence of Rh(D)-mediated HDFN has dramatically dropped. However, a gap between high-income countries and middle- to low-income countries was created largely due to a lack in availability and high costs of RhIG. The frequency of Rh(D)-negativity is highest in the Caucasian population (15–17%) and 8% in the Black population, whereas <1% of (East) Asian people are Rh(D)-negative. Unfortunately, little is known on the exact clinical and humanistic burden of the disease in sub-Saharan Africa, and data solely relies on estimates and case reports. Bhutani et al. estimated that almost 20,000/100,000 stillbirths and

40,000/100,000 neonatal deaths due to kernicterus occurred in sub-Saharan Africa in 2010.[103] This presents a striking contrast to the rarity of stillbirth and neonatal death in high-income countries and a great potential for improvements in epidemiological and clinical research on HDFN in these regions. We, and other researchers in the field, have therefore undertaken several projects in the region to address this major gap.

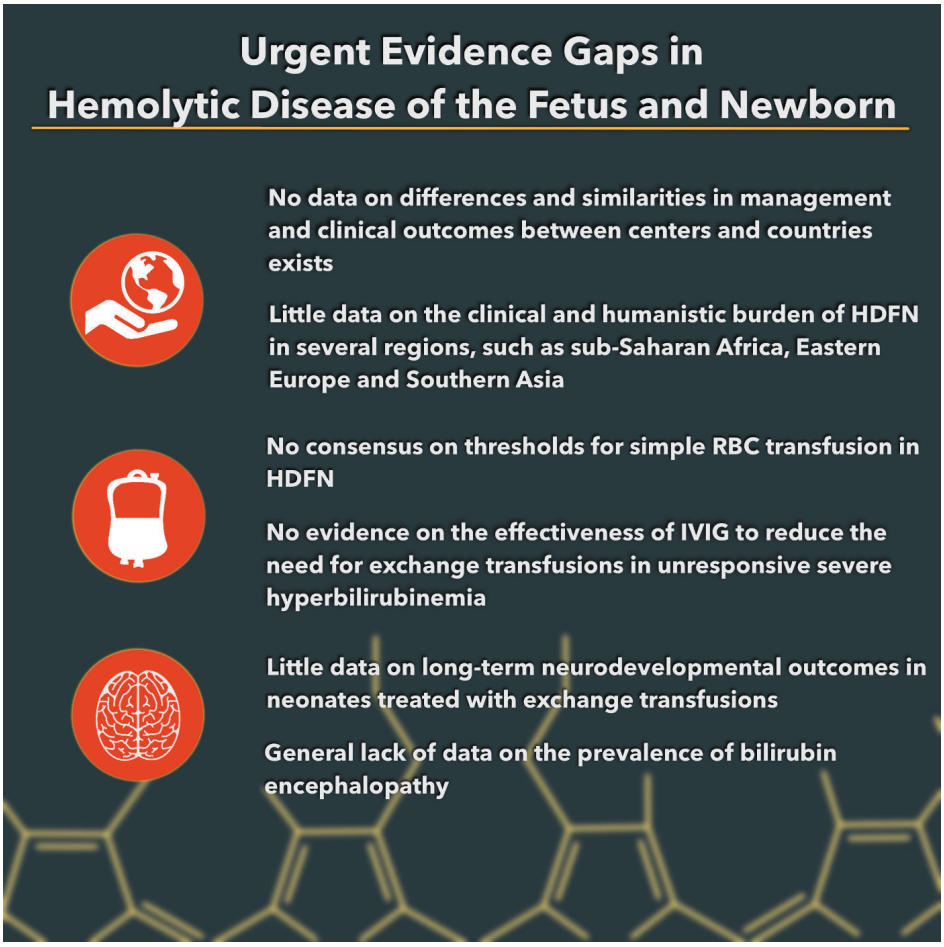

Figure 3: Urgent evidence gaps in hemolytic disease of the fetus and newborn. HDFN, hemolytic disease of the fetus and newborn; RBC, red blood cell

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