Optimising neonatal management of haemolytic disease of the foetus and newborn

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General discussion and future perspectives

Prevention and treatment of haemolytic disease of the foetus and newborn (HDFN) is generally considered a success story in perinatal medicine, as major progress has been made in the last decades in the understanding and management of this disease. Important steps in the management of HDFN include preventative and screening measures, including the availability of RhD immunoprophylaxis (RhIg)[1] and the development of completely non-invasive procedures to identify pregnancies at risk. Prevention and screening have impressively lead to a near disappearance of the severe complication of hydrops in the Netherlands.[2] Furthermore, improvement of the technique of intrauterine blood transfusions (IUTs) has raised the survival rate after IUT in experience centres to approximately 96%.[3] Postnatally, improvement of management and phototherapy has led to a drastic decrease in exchange transfusion rate (Chapter 4) and favourable long-term outcomes (Chapter 8).[4,5]

The aim of this thesis was to add to the knowledge on the use and outcome of current therapy options in HDFN, to critically evaluate the current neonatal management of HDFN and to set a base for further improvement and individualisation of postnatal treatment.

Pathophysiology

In HDFN, maternal red blood cell IgG alloantibodies that are transported across the placenta lead to the destruction of foetal and neonatal red blood cells.[6,7] Maternal alloimmunisation to the RhD and K (Kell) antigens is the most common cause of severe HDFN, but alloimmunisation can be caused by more than 50 different red blood cell antigens[8], and can be triggered by previous incompatible blood transfusions or foetomaternal haemorrhaging during pregnancy. Foetal haemolysis will initially induce compensatory erythropoiesis, but this can be insufficient to compensate foetal anaemia. Severe anaemia leads to a hyperdynamic foetal circulation, causing cardiomegaly and accumulation of oedema (foetal hydrops) and ultimately intrauterine demise if left untreated. IUTs to the foetus may be indicated to correct anaemia.[6,7,9]

Predictors of severe disease

It is important to identify children at high risk for severe HDFN and a complicated postnatal course as early as possible. One of the factors associated with severe illness are IUTs. The exact effects of intrauterine transfusions (IUTs) with adult donor red blood cells are not known. Children with HDFN that were treated with one or multiple IUTs, have been reported to have lower reticulocyte counts at birth, highlighting the fact that transfusions may lead to hyporegenerative anaemia.[10,11] Treatment with IUTs is also associated with a higher number of

red blood cell transfusions after birth compared to children with HDFN not treated with IUT(s), suggesting that suppression of erythropoiesis may last for weeks or even months (Chapter 5).[12] An inhibiting effect of donor blood on the foetal and neonatal erythropoiesis has been postulated before[10], but this thesis showed that the foetal reticulocyte count exponentially declines over the course of consecutive IUTs, with near disappearance of foetal reticulocytes after two IUTs and demise of the natural compensatory erythropoiesis (Chapter 3).[13] This leads to prolonged postnatal anaemia and an increased requirement of red blood cell transfusions after birth, regardless of type of alloimmunisation.[12,13] The strong suppressive effect of red blood cell transfusions was limited to the erythropoiesis, as a similar decline was not observed in foetal leukocytes and platelets.[13]

Foetal and neonatal haemolysis also result in excessive bilirubin levels. During pregnancy, excessive bilirubin is already present in the amniotic fluid and the membranes, and may also be cleared because it can pass the placenta and is excreted by the mother. After birth, the haemolytic process continues, giving rise to often severe hyperbilirubinaemia as excessive bilirubin cannot be conjugated by the immature neonatal liver. In severe hyperbilirubinaemia, unconjugated bilirubin can cross the blood-brain barrier and can lead to bilirubin neurotoxicity if left untreated. The clinical spectrum of bilirubin neurotoxicity is defined as bilirubin-induced neurologic dysfunction (BIND), of which kernicterus, or chronic bilirubin encephalopathy, is known as its most severe and permanent clinical manifestation.[14] Hyperbilirubinaemia is treated after birth with phototherapy or, if necessary, with exchange transfusions to lower bilirubin levels postnatally.[6,9]

Although much is known about bilirubin metabolism in newborn children, foetal bilirubin metabolism remains elusive. The immature, foetal liver is poorly capable of conjugating and excreting bilirubin.[15] Animal studies show that bilirubin can diffuse freely over the placenta, where it is conjugated in the maternal liver and excreted.[16,17] The (possibly harmful) effect of high foetal bilirubin levels on the foetus is unclear, as well as foetal bilirubin reference values. This thesis provides an overview of foetal bilirubin values as measured before the administration of IUTs, it showed that these values are of predictive value for the treatment course after birth. High foetal bilirubin values are associated with a higher chance for treatment with exchange transfusion. A foetal bilirubin cut-off value of 5 mg/dL at the last IUT has the highest sensitivity and specificity in terms of accurately predicting treatment with exchange transfusion after birth (Chapter 2).[18] Such predictors can help parents and caregivers to anticipate for severe illness after birth.

Neonatal management and complications

As mentioned, postnatal care consists of intensive phototherapy and exchange transfusions to treat severe hyperbilirubinaemia and top-up red blood cell transfusions to treat anaemia.

Since its introduction in the 1970s, phototherapy has been the corner stone of treatment of neonatal hyperbilirubinaemia and drastically decreased the necessity of exchange transfusion.[19] Phototherapy is generally considered a safe and very effective procedure (when started timely and efficiently), with very view reported adverse effects. Phototherapy lamps with an emission spectrum of 460-490 nm have proven to be the most efficient to lower the plasma bilirubin level by photo isomerisation of bilirubin in the skin to water-soluble isomers that can be excreted by the kidneys and stool without further conjugation by the liver. As a light source, LEDs (light-emitting diodes) have now mostly replaced fluorescent tubes and halogen spotlights.[9] An exchange transfusion is still indicated if phototherapy fails to effectively lower bilirubin levels, although guidelines have changed over the years on the definition of phototherapy failure and therefore on the timing of exchange transfusion after birth. Overall, the use exchange transfusions has been restricted and exchange transfusions are now recommended by the American Academy of Pediatrics (AAP) if bilirubin levels remain above exchange transfusion thresholds despite intensive phototherapy, or if signs of acute bilirubin encephalopathy occur.[20]

This thesis gives an update on the exchange transfusion use in children affected by HDFN in a Dutch tertiary care centre over the last twenty years. In line with other studies, a marked decline of exchange transfusion was observed (Chapter 4).[4,21-23] The incidence of exchange transfusion treatment declined from 67% for children born between 2000 and 2006, to 22% for children born between 2006 and 2015, and further down to 10% in children born more recently between 2015 and 2021. Improved intrauterine treatment procedures and postnatal treatment guidelines induced an increase in the time to a first exchange transfusion after birth from 6 to 50 hours. We observed that the strong decline in incidence of exchange transfusion and thus decreased exposure and expertise with this complex procedure, was not associated with an increase in procedure-related complications.[4]

Neonatal care also includes the management of postnatal anaemia that extends the first week after birth. Anaemia in children with HDFN is due to ongoing haemolysis by persisting and only gradually declining maternal antibodies, but also due to depressed erythropoiesis (hyporegenerative anaemia).[13,24] This thesis differentiated the postnatal transfusion need between children treated with and without IUT and analysed the time-dependent pattern of

anaemia and transfusion in these children. Of children affected by HDFN that were treated with one or more IUTs, 88% is in need of one or more blood transfusions after birth and the first transfusion was administered 16 days after birth. In comparison, 60% of children that did not require IUT were in need of one or more blood transfusions and the first transfusion was administered 9 days after birth (Chapter 5). Only 3% of children affected by HDFN show a transfusion dependency beyond 45 days after birth.[12] Several potential predictors for anaemia and postnatal transfusion-need were identified among children treated with IUT: a higher risk for D immunisation compared to K immunisation and, independent of the type of immunisation, a lower reticulocyte count at birth, whereas additionally a protective effect of exchange transfusion was observed. In children not treated with IUT, these predictors were: higher reticulocyte count at birth, higher peak levels of bilirubin after birth and also a protective effect of exchange transfusion.[12] These predictors can potentially be used to create individual risk profiles in order to fine-tune and individualise postnatal care.

The mechanisms behind late or hyporegenerative anaemia are unclear. It occurs in particular in children treated with several IUTs[11,13,25], but other contributing factors have been reported such as severity of HDFN and the declining use of exchange transfusions (hence less removal of maternal alloantibodies from the neonatal circulation).[4,9,12] Finally, erythropoietin (EPO) deficiency is also considered as a possible contributing factor to postnatal anaemia.[26-31] It has been postulated that there is an insufficient response in increase of EPO levels, and exogenous EPO has been increasingly used in full-term and preterm children to prevent or reduce neonatal anaemia without short or long-term adverse effects.[26,31-33] Several small studies and casuistic reports suggest that children with HDFN may benefit from treatment with EPO to reduce the risk of anaemia and subsequent transfusions.[26-31] However, other authors found that EPO may be less effective than expected.[35] Due to the lack of evidence, routine use of EPO is currently not recommended. To determine a role for administration of EPO in this group of patients, a randomised controlled clinical trial was designed to evaluate the effect of exogenous EPO on the prevention of postnatal anaemia in HDFN, as potential alternative for red blood cell transfusions. The protocol of this trial, the EPO-4-Rh trial (NCT03104426), is part of this thesis (Chapter 6). In this trial, children are randomised between conventional treatment (weekly haemoglobin measures and, if necessary, a red blood cell transfusion) and conventional treatment combined with weekly home visits and a weekly subcutaneous injection of recombinant human erythropoietin (in the form of darbepoetin alfa/Aranesp®). Potentially, EPO drives production of erythropoiesis leading to stabilisation of the haemoglobin levels of these children. EPO administration may thus prevent occurrence of late anaemia, hospital admissions for transfusions and potential transfusion reactions, creating a more stable and

natural postnatal course for patients with HDFN. In this scenario, the current management of weekly out-patient visits and weekly blood draws for haemoglobin level measurements, may be reduced, further contributing to reduction of the burden for these children. Patient recruitment commenced in October 2017, currently 43 of the intended 44 participants are included. The final results of this study are expected in the spring of 2022.

Aside from the well-known complications of hyperbilirubinaemia and anaemia in HDFN, other complications have been described such as thrombocytopenia and cholestasis. [36,37] HDFN has also been associated with the occurrence of necrotising enterocolitis.[38,39] Necrotising enterocolitis (NEC) is a severe gastrointestinal condition that can occur in newborn children and is defined by ischaemic necrosis of the intestine with a high mortality rate.[40] Although the risk to develop NEC is typically the highest among premature children and children with (very) low birth weights, NEC can develop as a complication among otherwise predisposed children.[41] This predisposition is likely an interplay of hypoxic-ischaemic injury of the gastrointestinal tract, physiological immaturity of the gastrointestinal tract and of the immune system, and alterations in the normal microbiological flora of the intestine.[42] Several of these predisposing factors for NEC are present in HDFN affected children, as HDFN can influence peripheral oxygenation and may also influence the gastrointestinal system because of high bilirubin levels. This thesis reports on the occurrence of NEC and its various clinical characteristics in a large population of children affected by HDFN. During the 17-year study period a relative risk to develop NEC for children with HDFN was found of 3.40 (95% CI 1.09-10.63), although the absolute risk of developing NEC in our HDFN cohort was low (1.3%) (Chapter 7).[43] These results emphasise that the HDFN population should be concerned as a distinct entity of the NICU population, with potentially distinct risk factors contributing to the development of NEC. Especially in otherwise predisposed children by late prematurity or a low birth weight, caregivers should be aware of the risk to develop NEC.

Long-term outcome

HDFN is nowadays a rare condition and the focus in management of HDFN switched from mere survival to optimal long-term outcomes. Long-term follow-up studies of children affected by HDFN are rare, and only a few studies with small patient numbers have reported on long-term neurodevelopmental outcome after IUT, but show overall positive outcomes. The outcomes measures of these studies are mainly survival rate and gross motor function and often included (very) severe cases of HDFN, complicated by foetal hydrops.[44-50] An earlier large cohort study in our centre found that the incidence of neurodevelopmental impairment (NDI) after IUT

was 4.8% (14/291), slightly higher compared to Dutch norm data. This study was conducted among children born between 1988 and 2008.[51] As foetal and neonatal management of HDFN has continued to develop after 2008, this thesis reports on a new evaluation of long-term outcomes of HDFN treated with IUTs. Outcomes were assessed specified to school performance scores as compared to Dutch norm data and behavioural functioning as reported by parents or caregivers and teachers. Data was reviewed for children between 6 and 12 years of age and showed a favourable and reassuring school development in children treated with IUTs. In the Netherlands, this treatment is centralised in the Leiden University Medical Centre (LUMC) in Leiden, a national referral centre for severe HDFN and foetal-therapy centre, guaranteeing a high level of experience. This study showed a normal distribution in school and behavioural development for live-born children with this condition, treated at the LUMC. The study showed that no children attend special-needs education and a quarter of children received or receive a (mild) form of additional support in school. When comparing school performance levels for reading comprehension and spelling, the study population had a similar distribution in scores compared to Dutch norm data. While the incidence of behavioural problems as reported by parents was similar to the Dutch norm data, teachers reported less behavioural difficulties in the study group (Chapter 8).[5]

Future perspectives

As mentioned, the understanding and management of HDFN have vastly improved over the last decades. Nevertheless, there are still major challenges to overcome as we strive to improve the management of HDFN.

One of these challenges is the great inequality of care for mothers and children affected by HDFN worldwide. HDFN continues to be a significant health problem in low-income and middle-income countries, affecting annually more than 150 000 children.[52] Before any other progress is made, available methods of prevention such as typing for the D antigen in pregnancy and RhD immunoprophylaxis should become globally available, since this will have major impact on reduction of HDFN worldwide. Pregnancies and children at risk need to be identified as early as possible in pregnancy, to enable timely treatment. In the Netherlands, introduction of routine first-trimester antibody screening in 1998 has highly contributed to the near-disappearance of hydrops, as the hydrops rate declined from 39% before 1998 to 15% afterwards and has been lower than 1% in more recent years.[2] Pregnancies complicated by alloimmunisation require specific supervision and, if necessary, timely (antenatal and neonatal) therapeutic intervention by an experienced medical staff.

In more developed countries a major challenge is, paradoxically, the increasing rarity of HDFN. The Netherlands are an example of centralisation of care for HDFN, where the LUMC acts as a national referral centre for severe HDFN. This setting allows the medical staff to uphold experience in complicated procedures as IUTs and exchange transfusions, but also gives an homogenous collection of data and follow-up records for these children. Despite our best efforts, data on HDFN is not complete, as milder cases of HDFN are treated at local hospitals rather than centralised to the LUMC. Nation-wide datasets on all HDFN cases should be pursued to provide broader insights in the whole spectrum of this disease. Furthermore, international collaboration is highly recommendable to obtain sufficiently sized patient cohorts to address the remaining questions regarding HDFN and ensure adequate enrolment of research trials. For this purpose, the start of an international registry for HDFN, the 'Dyonisus study', was recently initiated by our study group to collect and combine all available data for this increasingly rare disease. With this first international benchmarking study, we will evaluate and compare the differences and similarities in antenatal and postnatal management and (short-term and particularly also long-term) outcomes between all participating centres. Currently 15 international expertise centres involved in the care of children affected by HDFN are enrolled in this registry, while another 15 centres are at various stages of admittance.

A potential "game-changer" in the antenatal management of HDFN is nipocalimab, a monoclonal antibody designed to inhibit the activity of the placental IgG transport receptor, the neonatal IgG-Fc receptor (FcRn). FcRn actively mediates the transfer of maternal IgG across the placenta to the foetus, including pathogenic IgG causing HDFN. By inhibiting the function of FcRn, the transfer of IgG and also of anti-red cell alloantibodies might be prevented.[53] FcRn is not only important for placental IgG transport, but also has an important function in IgG level homeostasis in a person and in the recycling of albumin.[54] Given these functions of FcRn, safety considerations regarding anti-FcRn therapies include reduction in serum albumin levels, lowering of IgG levels in the pregnant woman and hypogammaglobulinaemia in the child due to blockage of IgG transport. Hypogammaglobulinaemia could increase the risk of infection, and the (long-term) immunological effects on the developing foetus and newborn child are unknown.[54] Recently, an internationally phase 2 trial called the UNITY-trial (NCT03842189) was initiated, to evaluate the potential of weekly intravenous nipocalimab infusion to delay or prevent the need for IUT in pregnant mothers at risk of early-onset HDFN prior to 24 weeks. Simultaneously, the CLARITY-study (NCT03755128) is an observational study to evaluate the clinical course and outcomes of pregnancies affected by early onset severe HDFN. These studies should help to unravel a potential role for nipocalimab in the treatment of HDFN.

For the future, important questions with regard to the use of nipocalimab or other type of FcRn inhibiting drugs remain to be answered, including the long-term effects of IgG depletion, timing of treatment and the benefit of nipocalimab in milder cases of HDFN that require no intervention, or only phototherapy.

With regard to treatment of HDFN, an interesting and ongoing discussion is also that of transfusion thresholds for newborn children. There is no consensus regarding optimal haemoglobin thresholds for transfusion and transfusion policies between centres and countries vary greatly.[55] An increasing number of studies suggest that restrictive transfusion guidelines may be preferable to liberal guidelines.[55,56] Perhaps even lower transfusion thresholds can be safely applied in this population, and the number of postnatal red blood cell transfusions decreased. This would significantly lower the postnatal burden of HDFN, as postnatal red blood cell transfusions are now the primary indication for readmission to the hospital for children with HDFN.

Another point of interest is the blood product used for IUTs and top-up transfusions, which may have major impact on foetal erythropoiesis and the hypogenerative anaemia seen in IUT-treated newborns. Foetal red blood cells predominantly contain foetal haemoglobin (HbF). Towards the end of pregnancy, the concentration of HbF is gradually replaced by adult haemoglobin (HbA). HbF comprises of 60 to 80 percent of total haemoglobin in the full-term newborn.[57] Nonetheless, IUTs and red blood cell transfusions in newborn children consist of irradiated adult red blood cells. Compared to HbF, HbA has a lower oxygen affinity, as HbF in the foetus has to take up oxygen in the relatively hypoxic intrauterine environment. It is not known how replacement of red blood cells containing HbF with red blood cells containing HbA, influences the erythropoiesis and perhaps also the production of erythropoietin. Finally, foetal red blood cells also have a shorter life-span than adult red blood cells of which the physiological consequences are unknown.[58] The use of umbilical cord blood, with predominantly HbF, is widely established for haematopoietic stam cell transplantation, but has been used as well for transfusion purposes in research settings.[59] Although no report of adverse transfusion effects have been seen, matters of concern are the limited availability of umbilical cord blood and a relatively high reported bacterial contamination rate.[60]

Aside from medical-technical questions that remain to be answered, the social and emotional burden of HDFN on parents and caregivers should never be underestimated. From personal communications with parents and caregivers, we learned the great uncertainty and anxiety that can arise already early in pregnancy if HDFN is diagnosed. HDFN is rare and

pathophysiological sometimes very complex to comprehend, which can lead to a feeling of isolation, with little understanding by the family's surroundings. These pregnancies are also time-consuming in terms of follow-up, especially as parents and caregivers have to travel far to the LUMC or other specialised foetal-therapy centres. Additionally, increasing rareness of this disease and scattered antenatal and neonatal care, may result in insufficient knowledge among health professionals and subsequent inadequate information transfer to parents and caregivers confronted with HDFN. Dutch obstetric care providers, for example, showed a general lack of knowledge on HDFN.[61] Actively, frequently and correctly informing parents and caregivers, through all stages of pregnancy and early parenthood, and if necessary by referral to specialists in this field, should always be a main concern in the management of HDFN. A prerequisite here is to carefully balance efforts to decrease the medicalisation of the postnatal course of HDFN while guaranteeing a safe environment in which parents and caregivers do not feel overwhelmed by a responsibility of being the main observer and decision maker on the well-being of their child, with only professional evaluation if asked.

The future management of HDFN also advocates for a more individualistic patient approach, rather than treating children with HDFN as a homogenous group. It is for example unclear why two children with equally high levels of bilirubin can have a very different neurological outcome, or why children with equal antibody titres can have a completely different postnatal treatment course with regard to need for exchange transfusion or red blood cell transfusions. This thesis added more potential predictors of severe HDFN with a complicated postnatal course to what was already known.[12,13,18] Ideally, these can be compiled with genetic factors to eventually build individualised risk profiles for children affected by HDFN to pinpoint postnatal follow-up and treatment to those most at risk. Important factors to further look into are for example the value of antibody-dependent cellular cytotoxicity (ADCC)-tests and other antenatal tests as predictors of a complicated postnatal course, to guide postnatal care as early and efficient as possible. As HDFN becomes further individualised, centralisation of care for the high-risk population, remains vitally important to cultivate the current high standard of care and improve upon this care in the future.

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