Hemolytic disease of the fetus and newborn: awareness precedes change

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PART VII

DISCUSSION

General Discussion And Future Perspectives

GENERAL DISCUSSION

Over the past century, numerous developments and advancements have improved the survival rate and patient outcomes for hemolytic disease of the fetus and newborn (HDFN). The resulting rarity of severe HDFN has challenged our ability to study and improve various aspects of the care continuum, including screening, risk-based laboratory testing, and antenatal and postnatal management. Additionally, the absence of continuous active exchange of data has led to a lack of up-to-date information on management practices across centers worldwide. Finally, there was a prevailing belief in fetal-maternal and neonatal medicine that the management of HDFN had reached its optimal level. However, fostering a culture of continuous improvement remains crucial as it is our responsibility to provide the highest standard of care available. This thesis challenges our current understanding and provides recommendations for the antenatal and postnatal management of HDFN, aiming to improve patient outcomes and offer insight into novel developments that may be of future benefit.

Refining the diagnosis and identification of at-risk pregnancies

HDFN is caused by the destruction of fetal and neonatal red blood cells (RBC) induced by maternal alloantibodies directed against paternally inherited blood group antigens, most commonly RhD, other Rh-antigens, and K1 (Kell). Early identification of pregnancies with RBC alloantibodies together with timely referral of pregnancies at risk of fetal anemia is pivotal to prevent fetal hydrops and fetal death. Especially considering that the presence of fetal hydrops is strongly associated with adverse long-term neurodevelopmental outcome as shown in previous research[1] and in **Chapter 14**. Accurate identification of all pregnancies that require an intrauterine transfusion (IUT) (i.e., 100% sensitivity) whilst limiting the referral of pregnancies that do not require an IUT (i.e., false positive rate) is crucial to prevent adverse outcome on both ends of the spectrum. Classically, serial serological monitoring through the determination of the antibody titer in maternal serum is performed to bring about this policy. The antibody titer has been used internationally in the past decades, however, sole use of the antibody titer is struck with a relatively high false positive rate. To mitigate this whilst maintaining a 100% sensitivity, the Netherlands investigated and implemented the antibody-dependent cellular-cytotoxicity (ADCC) assay. In this comparative study, the authors found a lower false-positive rate of 39% at ADCC values of 50% or above, compared to a false-positive rate of 77% when using the antibody titer at a critical value of 1:16.[2] But, despite its strong predictive ability, the ADCC has its drawbacks as it is costly, labor-intensive, requires pooled monocytes from a large number of volunteer donors and is dependent upon radioactive material. As such, the ADCC

is not used in other countries than the Netherlands, and the international need for a simplified and accurate test is of paramount importance.

Chapter 10 describes our efforts in approaching this need. In line with previous research, we found that IgG-Fc-fucosylation levels are lower in pregnancies with IUT compared to pregnancies without IUT. However, we established that IgG-Fc-fucosylation as a sole determinant could not be employed in clinical practice. Fetal hemolysis is a complex process that depends upon many factors, such as antibody quantity, IgG subclass, transport efficiency, possible interfering of inhibiting HLA-class II antibodies, IgG-Fc-glycosylation profiles and fetal IgG-Fc receptor profiles. The novel flow-cytometric assay includes these factors and preliminary results showed that the false-positive rate could be considerably limited whilst maintaining a 100% sensitivity, compared to standard-of-care. Considering the study's limitations, confirmation of these findings with serial determination in a new prospectively collected cohort without selection bias is required. This should be focused upon by researchers, clinicians, and companies as the potential wide-spread use of FcRn-inhibitors to prevent fetal anemia and the need for IUT, further touched upon below, will likely limit our capability to develop new assays to identify pregnancies at-risk.

Antenatal management

The antenatal management of HDFN focuses on the early identification of fetal anemia through non-invasive Doppler sonography. A key component to this is prompt referral of pregnancies at risk of fetal anemia to prevent fetal hydrops, as fetal hydrops is linked to poorer short-term and long-term outcomes. In **Chapter 4** we describe considerable variations in referral strategies among 31 international centers. Whereas some centers primarily have patients referred upon the serological identification of alloimmunization, other centers primarily received patients based on a sonographically suspected fetal anemia. Numerous factors may underlie these differences, such as a lack of consensus on the optimal referral strategy, geographic challenges and the (un)availability of materials. Despite underlying differences between centers, efforts should focus on minimizing the incidence of fetal hydrops, using its occurrence as a key indicator to help inform and refine referral strategies. Developments to optimize and simplify the identification of pregnancies at risk of fetal anemia as described in **Chapter 10** may help to overcome these challenges and aid in preventing fetal hydrops whilst also limiting the number of pregnancies with unnecessary sonographic monitoring. Increasing awareness among clinicians, institutions, and regulatory bodies (e.g., ministries of health) on the importance and potential added value of early detection and management of pregnancies at risk of fetal anemia due to HDFN is of paramount

importance. Considering differences across regions and between countries, this ought to be done in context-specific settings.

A subset of pregnancies may develop fetal anemia that is managed by fetal blood sampling and providing an IUT. IUTs are the cornerstone of treating fetal anemia to bridge the gap to a viable gestational age for delivery, and often multiple IUTs are required. The procedure is regarded safe in experienced hands, but is technically challenging and is associated with risks inherent to its invasive nature. In **Chapter 4** we established that there was an overall preference to perform IUTs in the umbilical vein at the placental insertion, followed by intrahepatic transfusions in the hepatic vein. However, we also found a wide variation among centers. We hypothesized that this variation could largely be explained by previous training and personal preferences. This hypothesis was later supported by a Delphi consensus study that found that most North-American specialists tended to use the placental insertion whereas European specialists tended to use the intrahepatic approach.[3] Studies to optimize IUTs and limit the complication rate are challenging and largely depended on single-center experiences owing to the rarity of the procedure.[4-10] Previous research of more than 1600 IUTs performed at the LUMC found that the use of fetal paralysis, avoidance of arterial puncture and the use of an intrahepatic approach were associated with a lower likelihood of complications.[4] The DIONYSUS study enabled us to further elaborate on complication rates per fetal access site in a multicenter context. Similarly, we found that the intrahepatic approach, with or without an intraperitoneal deposition, was associated with a lower likelihood of non-lethal complications compared to IUTs performed in the placental insertion or in a free loop of the umbilical cord, providing further evidence in support of intrahepatic transfusions.

It can also be deduced from the study's findings that some centers may choose to perform a transplacental IUT, considering the equal distribution of anterior and posterior placentas. Compared to paraplacental approaches, transplacental IUTs carry a higher risk of transplacental hemorrhages, are associated with an increase in antibody titer, and are associated with an increased Odds in the formation of new alloantibodies directed against other blood group antigens which may complicate current and future pregnancies.[11-16] Studies to identify factors that determine whether a specialist may perform a trans – or paraplacental IUT, such as training, gestational age and position of the placenta, is warranted.

Like previous studies, we also found an increased risk for fetal death following IUT in procedures performed at early gestations[4,17,18], specifically 3.3% before 23 weeks' gestation compared to 0.4% at or after 23 weeks' gestation. Several studies have

been performed to study the effectiveness of weekly intravenous immunoglobulin (IVIG) administrations, with or without plasmapheresis, to delay or prevent fetal anemia in pregnancies with a history of early onset HDFN (fetal or neonatal death due to HDFN or a history of IUT before 24 weeks) in order to delay the need for the first IUT thereby mitigating the risks associated with early IUTs.[19-23] However, contrary to what is reported in literature, we report in **Chapter 4** that a majority of pregnancies treated with IVIG with or without plasmapheresis did not have such an obstetric history. This may be due to a lack of consensus and limited evidence, as most studies on these treatment options lack robustness or may not be applicable to current practice, given that many were published before the wide-spread use of Doppler sonography to detect fetal anemia. The PETIT-study, a multicenter, retrospective cohort study published in 2018 evaluated the effectiveness of 1g/kg weekly IVIG administrations on delaying or fetal anemia in consecutive pregnancies of women with a history of early onset severe HDFN.[19] The authors found IVIG, especially if started before 13 weeks' gestation, could delay the onset of fetal anemia by an unadjusted 15 days and statistically adjusted 4 days.[19] These findings, including a lower rate of fetal hydrops and a higher rate of live birth were recently confirmed in a multicenter individual patient data meta-analysis.[24] The DIONYSUS study's finding of a large variation in the use of IVIG, and plasmapheresis, is also underlined by the Delphi consensus study from Mustafa *et al*. that reported that the majority, but still only 70% of experts agreed on using IVIG in patients with a history of early onset severe HDFN.[25]

Optimizing the timing of delivery

Over the past decades it was common practice to deliver affected neonates at approximately 36 weeks' gestation to limit the effects of increasing IgG transport at increasing gestations, thereby theoretically limiting the postnatal disease severity. In **Chapters 2 and 4** we indeed found that most neonates were born at late preterm gestations, particularly those that experienced fetal anemia and required IUT during pregnancy. However, we also found a striking variation in gestational age at delivery among participating centers as described in Chapter 4, with some centers averaging around 34 weeks' gestation and others averaging at or after 37 weeks of gestation, suggesting differences in guidelines and perspectives in the timing of the last IUT and subsequent delivery. Considering that preterm delivery occurs at a crucial time in fetal development, the consequences may have long-lasting and potentially detrimental effects, even in late preterm delivery. Compared to term gestations (37 weeks or above), late preterm delivery (34 to 36 weeks' gestation) has been associated with lower cognitive scores, poorer school performance, more frequently reported behavioral problems, and ongoing morbidities.[26] Considering this, we aimed to investigate the effects and potential

of a policy of term delivery compared to the standard of care policy of preterm delivery. We hypothesized that a delivery after 37 weeks in HDFN is associated with an increased fetal maturation, a decreased postnatal disease severity, improved long-term outcomes and in particular a reduced need for exchange transfusions.

We highlighted this in **Chapter 5** where we described our findings on the effects of gestational age at delivery on the exchange transfusion frequency and on the likelihood of adverse neonatal outcome. Firstly, after excluding neonates with potential confounding variables, such as antenatal or postnatal IVIG, we found that a higher gestational age at birth was associated with a decreased exchange transfusion frequency, decreasing from approximately 38% at a gestation of 34 weeks to approximately 17% in after 37 weeks. This finding was consistent in both neonates with IUT and neonates without IUT. Secondly, we devised a composite outcome to assess and quantify the effect of independent variables on short-term neonatal morbidities. The composite outcome, neonatal adverse outcome, consisted of respiratory distress syndrome, necrotizing enterocolitis at Bell stage 2A, culture-proven bacterial sepsis, severe cerebral injury, kernicterus, and neonatal mortality. We found that each weekly increase in gestational age at birth was associated with a 43% decrease in the likelihood of adverse neonatal outcome, with considerably less morbidities among neonates born at or after 37 weeks gestation.

Then to further substantiate our findings, we sought to investigate the effect of gestational age at birth, among other variables, on the long-term neurodevelopmental outcome of children with IUT in **Chapter 14**. In this cohort study of nearly 400 children managed over a period of 35 years we found that every additional week in gestational age at birth was associated with an approximately 30% decrease in the likelihood of neurodevelopmental impairment, that was defined as a composite outcome consisting of cerebral palsy ≥GMFCS level II, severe cognitive developmental delay (cognitive scores below 2 standard deviations), hearing loss or deafness and bilateral blindness.

However, delivery at or after 37 weeks may be complicated by fetal anemia occurring at late gestations. It has been common practice to refrain from performing IUTs in fetuses with suspected fetal anemia past a gestation of 35 weeks. Instead, induction of preterm delivery or a planned cesarean section was commonly performed in those instances, considering the potential risks associated with invasive fetal blood sampling and IUTs. However, in **Chapter 4** we have shown that the risk of fetal death due to IUTs performed after 32 weeks to a maximum of approximately 37 weeks is minimal and that the rate of non-lethal complications is similar across all gestational ages. Given

these findings, we believe that IUTs after a gestation of 35 weeks is a viable treatment option and should be strongly considered to bridge the gap to delivery after 37 weeks.

Taken together, these findings support a policy of waiting for delivery until after 37 weeks of gestation, contrary to the standard of care of late preterm delivery.

Postnatal management

Hyperbilirubinemia – Intensive Phototherapy

After delivery, neonates are struck with the consequences of continued hemolysis, owing to circulating pathogenic maternal IgG, and an impaired erythropoiesis in case of K1 (Kell)-mediated HDFN or if any IUTs were administered during pregnancy. During pregnancy, fetal bilirubin that is released in fetal circulation due to the destruction of fetal RBCs is actively transported across the placenta and conjugated and excreted by the pregnant woman. After delivery, however, neonates must conjugate and excrete bilirubin themselves, without the help of their mother. Considering their fetal immaturity and increased exposure to bilirubin postnatally, these neonates may experience a certain level of hyperbilirubinemia particularly in case of increased hemolysis such as in HDFN. Intensive phototherapy is the first-line treatment for hyperbilirubinemia, owing to its easy accessibility and non-invasive method of treating hyperbilirubinemia by transforming unconjugated bilirubin into water-soluble bilirubin photoisomers.

The fact that phototherapy serves as the ubiquitous first-line treatment is underlined by the finding that 94% of 1855 neonates reported upon in **Chapter 5** received phototherapy. Among those, complications associated with phototherapy occurred in less than 2% and concerned mild adverse events only further underlining its value in clinical practice. However, in some cases the severity of hyperbilirubinemia may increase despite intensifications of phototherapy, and exchange transfusions may be required.

Hyperbilirubinemia – Exchange Transfusions

Since its introduction, exchange transfusions played a pivotal part in the management of severe hyperbilirubinemia. However, this risky procedure became increasingly rare following developments to prevent severe hyperbilirubinemia, through for instance early identification of hyperbilirubinemia and application of intensive phototherapy. A cohort study performed at our center on neonates with HDFN admitted to the NICU uncovered that the incidence of exchange transfusions had considerably dropped from 67% in the years 2000-2005 to 10% in 2015-2020.[27] Although this single-center study found no increase in procedure-related complications,[27] the clinical experience with this complex procedure may have declined, resulting in fewer pediatricians being

equipped to perform exchange transfusions. Additionally, there is no data available on the actual prevalence of exchange transfusions and its indications on a national scale. Enabled by the centralization of the national blood supply in a single collection and distribution agency, we devised a multicenter, retrospective cohort study to retrieve data on the use of exchange transfusion blood products (c.q. reconstituted whole blood exchange transfusions).

Firstly, in **Chapter 7**, we observed a rising frequency of exchange transfusions corresponding to higher maternal serological titers in neonates with D-mediated HDFN. Among 85 neonates who underwent exchange transfusions for D-mediated HDFN, the rate increased from approximately 1% when titers were at or below 1:32 to about 20% when titers reached 1:512. An important finding, specific to the Netherlands, was that the ADCC could relatively well distinct between 'low risk' cases and 'high-risk' cases: exchange transfusion rates increased from approximately 1% if the maximum ADCC was below 50% to approximately 19% if the ADCC was at or above 50%. The application of these findings could aid in further improving care for our patients on an individual basis by determining the optimal place of birth and aid caregivers in anticipating severe hyperbilirubinemia. The necessity for an antenatal risk estimation was further substantiated by the questionnaire's findings. Among 49 Dutch centers without a neonatal intensive care unit, we found that fewer than 40% currently perform exchange transfusions, a significant decline from 80% prior to 2010, suggesting a loss of expertise on a national level owing to the procedure's rarity and a shortage of healthcare professionals and materials as reported by the centers themselves.

Then, in **Chapter 8**, we delved deeper into the indications for exchange transfusions and use and waste of reconstituted whole blood exchange transfusions. Among more than 600 neonates for whom reconstituted whole blood exchange transfusions were ordered by 24 participating centers, we identified that most products were ordered for severe hyperbilirubinemia, and we found several rarer indications such as severe leukocytosis. Importantly, we also found that approximately one-half of the ordered reconstituted whole blood exchange transfusions were unused, especially in neonates with severe hyperbilirubinemia, owing to a successful decrease in bilirubin after an intensification of phototherapy. Overall, we found that the prevalence of neonates who received an exchange transfusion was approximately 15 per 100.000 liveborn neonates, indicating the rarity of this procedure. Additionally, most procedures were performed in neonatal intensive care settings: annually 31 neonates versus 5 neonates in the participating non-intensive care centers.

We expect that the implementation of the increased thresholds for exchange transfusions developed by the American Academy of Pediatrics and taken up in the revised 2022 guideline will further decrease the prevalence of exchange transfusions on a national scale. The LUMC has implemented these revised and increased thresholds in February 2025.

Considering the low prevalence of exchange transfusions, the rapid decline in the number of hospitals capable of performing exchange transfusions, the annual number of neonates with an exchange transfusion per type of center, and the potential of using maternal serological test results to anticipate exchange transfusions in HDFN, we advocate to regionally centralize exchange transfusions in (relatively) larger volume centers to ensure the availability of procedure-related materials and of trained staff.

Lastly, as mentioned in **Chapter 3** we found a large variation in the rate of exchange transfusions among published studies, suggesting that the frequency of this procedure could vary significantly between centers. This finding was substantiated in **Chapter 5** in which we report that exchange transfusion frequencies varied between 0% and approximately 80% between the 31 centers participating in the DIONYSUS-study, in both neonates with IUT and neonates without IUT. This vast difference between centers can only partially be explained by differences in case mix, but may also be due to differences in guidelines, differences in perspectives on when to perform an exchange transfusion, and differences in the application of phototherapy (e.g., number of lights available, double-sided application, correct distance, irradiance and wavelength, level of skin exposure). Nevertheless, we can only speculate on the exact underlying causes of this variation and further discussions among neonatologists are required to identify opportunities to unify care between centers.

Hyperbilirubinemia – Intravenous Immunoglobulins

Over the past decades IVIG has been used to prevent exchange transfusions among neonates with ABO or non-ABO HDFN. However, several randomized controlled trials and a Cochrane review failed to show a beneficial effect of providing IVIG at birth to prevent exchange transfusions.[28-37] Nevertheless, data on the effectiveness of IVIG in case of an impending exchange transfusion is not available, as summarized in **Chapter 1**. Despite the lack of robust evidence, the American Academy of Pediatrics describes IVIG administration of 0.5-1.0 g/kg as a viable treatment option to potentially delay or prevent an exchange transfusion in case of severe hyperbilirubinemia.[38] In **Chapter 5**, as part of the international DIONYSUS study we indeed found that 75% of participating centers used IVIG to prevent or delay an impending exchange transfusion. We also identified wide variations in the doses administered, with 35% of neonates receiving

doses exceeding the recommended 1 g/kg. This lack of consensus on whether to administer IVIG in such clinical situations is evidently due to the lack of evidence. Furthermore, among more than 429 neonates treated with IVIG, we only found 1 neonate who developed necrotizing enterocolitis, in contrast to a study reporting om 10 cases with necrotizing enterocolitis. Our study may aid in reducing neonatologist's hesitancy in administering IVIG in neonates with severe hyperbilirubinemia due to HDFN. Nevertheless, considering the risks associated with administering blood donor products, although minimal as was also shown in **Chapter 5**, and taking into account the costs associated with IVIG, we urge the field to address the need for a randomized controlled trial, or target trial emulation using retrospective data[39], to evaluate the effectiveness of IVIG in delaying or preventing an exchange transfusion in such situations.

Persisting anemia

During the first three months of life, infants with HDFN may experience a certain level of anemia owing to continued hemolysis and inhibition of erythropoiesis (hyporegenerative anemia). In fact, previous research has shown that nearly 90% of infants with IUT and 60% of infants without require at least one RBC transfusion, at a median of 2 RBC transfusions.[40] Monitoring for anemia, whilst also assessing the level of erythropoiesis by measuring reticulocyte counts, is essential to timely identify those that require a RBC transfusion and prevent potentially devastating consequences. However, there is no consensus on neither the monitoring frequency as on the hemoglobin thresholds at which a RBC transfusion is required and, consequently, large differences between centers may exist. This was touched upon in **Chapter 5** in which we identified wide differences in monitoring strategies ranging from "*in case of clinical symptoms"*, "*once a month"*, "*once per two weeks*", "*once per week*" to "*twice per week*". We also found a highly variable range in the frequency of RBC transfusions during initial admission among participating centers, ranging from approximately 45% to 100%. We were, however, unable to assess differences in the frequency of RBC transfusions owing to a lack of available data bearing in mind that most of the monitoring for anemia was done at other centers. Nevertheless, it is highly likely that we would also find differences between centers in that regard. Taken together, we advocate to aggregate data on RBC transfusions in neonates born at or after 35 weeks of gestation, consolidate knowledge in this field and reach consensus among neonatologists and transfusion medicine experts to provide advice on the monitoring strategy and hemoglobin thresholds for RBC transfusions. Ideally, the monitoring strategy is adapted to an individual basis by several variables such as the type of alloimmunization, reticulocyte count at birth and the need for exchange transfusion as studied by Ree *et al.*

Given that most infants, particularly those who received two or more IUTs, require at least one RBC transfusion, clinicians should remain mindful of the potential inhibitory effects of IUTs and RBC transfusions on the infants' erythropoiesis. This so called "hyporegenerative anemia" may result from an inhibitory effect of transfusions. The transfused RBCs containing adult hemoglobin have a lower affinity to oxygen than RBCs containing fetal hemoglobin and could suppress the infants' erythropoietin production. Based on this hypothesis, Ree et al. performed an open-label, single-center, randomized controlled trial to evaluate the effect of weekly subcutaneous administrations of 10 μg/kg darbepoetin alfa on the number of transfusion episodes compared to standard of care. The authors observed a significant decrease in the number of RBC transfusions, with a median of one RBC transfusion in infants treated with darbepoetin alfa compared to a median of two RBC transfusions in those receiving standard care. Additionally, they reported no short-term complications linked to darbepoetin alfa. To further inform clinical practice on the potential value of using darbepoetin alfa, we sought to assess the neurodevelopmental outcome of the children that participated in this trial as described in **Chapter 12**. Overall, the neurodevelopmental outcomes were favorable in both groups. We could not demonstrate improved neurodevelopmental outcomes in children treated with darbepoetin alfa compared to children with standard care, likely due to limitations of this post-hoc analysis. Taken together with the findings of Ree et al., we propose to include darbepoetin alfa in the arsenal of pediatricians, particularly for neonates with multiple IUTs and low erythropoietic activity, to reduce the number of RBC transfusion episodes. Further studies powered to evaluate the effect of darbepoetin alfa and fewer RBC transfusions on neurodevelopmental outcomes are required.

Long-term neurodevelopmental health

Monitoring long-term outcomes is essential to identify factors associated with adverse or favorable outcome to advance and refine clinical strategies. But, long-term follow-up, especially in HDFN owing to its limited extent to three months after delivery, is challenging. This was also apparent from the DIONYSUS-study, in which long-term neurodevelopmental outcomes was only available in less than 10% of neonates, with results significantly impacted by a high level of selection bias. As a result, we were unable to assess long-term outcomes or determine any associated factors in that study. To address that gap, we aimed to assess the long-term neurodevelopmental outcomes of children with IUT who were treated at our center in a retrospective cohort study. As described in **Chapter 13**, we found favorable outcomes among 365 children treated in a 35-year time period with a normal median full-scale IQ-score of 101, and neurodevelopmental impairment was found in 5% of children. Importantly, as was

described previously, we uncovered key factors associated with adverse outcome and our findings underline that pregnancies at risk of fetal anemia ought to be referred early to prevent fetal hydrops. Similar to the findings of the LOTUS-study (Long-Term follow up after intra-Uterine transfusionS)[1], we found that children with fetal hydrops at the first IUT were 3.4 times more likely to have neurodevelopmental impairment. We also found that each additional week in gestational age at birth was associated with a 30% decrease in the likelihood of neurodevelopmental impairment. This finding supports the recommendations of waiting for delivery until after 37 weeks. Additionally, these identified key factors could be used by caregivers to guide and personalize the inclusion of patients for long-term follow-up. In that case, follow-up should be offered to patients with severe fetal hydrops, and patients born before 37 weeks.

The way forward: Innovations and developments in HDFN

Tackling global disparities

Despite significant advancements in the past century, we have come to learn that there are large disparities across different regions of the world. While the prevalence of HDFN has drastically dropped in many high-income countries and the clinical outcomes of those affected are favorable when many resources are available, many middle- to low-income countries lack a screening and prophylaxis program, and often, there is limited access to equipment for timely monitoring and treating pregnancies, fetuses and neonates affected.[41] As a results, the prevalence and burden of HDFN is likely to be much higher in middle- to low-income countries. However, there is no robust epidemiological data on the actual burden of HDFN in regions such as South Asia and sub-Saharan Africa. It is estimated that more than 50% of pregnancies requiring RhIG do not receive it, particularly in middle- to low-income countries.[42] Furthermore, around 52,000 stillbirths and 74,500 cases of kernicterus occur globally each year.[43] As highlighted by *Bhutani et al.*, the combined mortality rate in Eastern Europe, Central Asia, Latin America, sub-Saharan Africa, and South Asia is 119 per 100,000 live births, a stark contrast to 1 per 100,000 live births in high-income countries.[43]

The shortage of data from Africa, South America, South Asia, and Eastern and Central Europe on the rate of RhD-negativity, the immunization rate to RhD in pregnancies, and the clinical burden of this largely preventable disease is a critical knowledge gap that needs urgent attention. Addressing this data shortage is essential to understand the global impact of RhD-mediated HDFN and to implement screening and prophylaxis strategies in these regions as called upon by the International Federation of Gynecology and Obstetrics and the Worldwide Initiative for Rh-Disease Eradication.[44] Among several other initiatives that followed, the AFRICARhE consortium was established to

foster collaboration to share knowledge between the Kilimanjaro Christian Medical Center in Tanzania, Queen Elizabeth Central Hospital in Malawi, Haramaya University in Ethiopia, and Sanquin and the Leiden University Medical Center in the Netherlands. The AFRICARhE consortium aims to address this major knowledge gap, and ultimately implement nationwide sustainable screening and prophylaxis programs and ensure that treatments for those affected by HDFN are available. Achieving this aim and ensuring that our patients receive optimal care on a global level is not only a responsibility of our consortium but is a global responsibility of healthcare organizations and health care professionals.

A striking finding was that a monoclonal RhIG produced by Bharat Serums and Vaccines is widely available across the African continent, in spite of a serious lack of evidence on its effectiveness in preventing RhD immunization.[45] Polyclonal RhD immunoprophylaxis, proven effective in multiple randomized controlled trials, has been widely available in high-income countries since the late 1960s. However, most African countries have little to no access to this essential treatment. As highlighted by Pegoraro *et al*., there is a significant shortage of polyclonal RhIG derived from volunteer plasma donations, which hampers the ability to supply those in need.[42] Regional volunteer plasma donations and regional production of polyclonal RhIG may help to overcome this challenge. Still, a near unlimited supply of effective monoclonal RhIG would be optimal. However, the production of monoclonal RhIG is challenging owing to our lack of understanding on the mechanism underlying RhD immunization in pregnancy, the absence of appropriate animal models[45], and the lack of uniform protocols for trials. The development of effective monoclonal RhIG to address the shortage of polyclonal RhIG is essential. To facilitate this, protocols and study requirements for evaluating the efficacy of monoclonal RhIG should be developed in collaboration with health care institutions and regulatory bodies, such as the European Medicines Agency, the United States Food and Drug Administration, and the African Medicine Agency. Ultimately, it is our responsibility as healthcare professionals to address inequity in healthcare and enhance the quality of medical care for all patients.

Reaching consensus

In **Chapters 4 and 5** we have shown a great variation between centers in the timing and reason for referral, presence of hydrops, timing of IUTs, techniques used in IUTs, use of IVIG administrations and plasmapheresis to delay fetal anemia, timing and method of delivery, exchange transfusions, use of erythropoiesis stimulating agents, IVIG to manage hyperbilirubinemia and RBC transfusions, along with highly variable guidelines. These studies have provided an invaluable opportunity to enable comparison of a centers' data against the performance of other centers. This capability is a crucial

first step to raising awareness, sparking meaningful discussions, and driving change. Furthermore, by offering insights retrieved in the DIONYSUS study, our research offers colleagues an opportunity to implement evidence-based improvements that lead to better clinical outcomes, such as the delivery of affected neonates at a gestational age of 37 weeks or later. Through these studies we have shown that the management of HDFN had not reached an *'as good as it gets'* state and that several aspects could be improved by retrieving data and learning from each other's experiences in an international setting. Reaching consensus on the identification, referral, antenatal management, delivery, and postnatal management, or addressing gaps in evidence if consensus cannot be achieved, is essential to unify practice across centers and countries, limit variations, and ensure that our patients receive optimal and evidence-based care. Following the DIONYSUS study, two expert Delphi survey studies were initiated by the fetal medicine community to determine consensus.[3,25]

Navigating the challenges of the General Data Protection Regulation

As we described in **Chapter 6**, multicenter, observational research is crucial to study low prevalence diseases, such as HDFN, to identify opportunities to improve the clinical outcomes of those affected. Through the DIONYSUS study and WISE study, we gained first-hand experienced in performing multicenter, observational research under the General Data Protection Regulation Act. These studies were challenged by the varying ethical applications and different interpretations of the GDPR, leaving us in stacks of paperwork, frustration and striking our research by high costs and inefficient use of time. In our commentary we referred to performing observational research under the GDPR as "*…trying to cross a busy street blinded by paperwork, with more forms handed to you at every step, and each crossing guard enforcing different rules. One says, 'Stop for a green light,' another says, 'Go on orange,' and a third insists you 'Wait for blue', even though such a light does not exist. All the while, you cannot shake the feeling that taking a nearby crosswalk would simplify everything.*"

Thanks to a dedicated PhD position, we were able to bring these studies to fruition. However, we do express our concerns, as such PhD positions are uncommon and many students, nurses and clinicians may lack the time or resources to initiate similar studies. To ensure the success of multicenter observational studies, it is of paramount importance to unify and simplify the ethical approval process within countries and across borders. Streamlining this procedure will enable a broader range of professionals to contribute to impactful research, foster (inter)national and multidisciplinary collaborations and drive advancements for our patients.

Socioemotional burden on parents

Interventions used in the management of HDFN primarily aim to mitigate the physical health consequences of the disease. However, these interventions may also impact the socioemotional well-being of families. Despite the clinical focus, the socioemotional impact of HDFN and its treatment, such as IUTs, planned delivery or exchange transfusions, remain unexplored and provide a valuable opportunity for research. Qualitative studies, including retrospective focus groups, in-depth interviews, and prospective diary studies or standardized questionnaires, could be used to provide insight stress and anxiety experienced by parents, the impact on parental relationships, and potential issues related to bonding and attachment.

Inhibiting the neonatal Fc receptor

Developments in the management of in pregnancies with fetal anemia throughout the years have led to a decrease in mortality and improvement of clinical outcomes. Nevertheless, pregnancies that develop fetal anemia and require IUTs at early gestational ages, particularly before 24 weeks' gestation, are at risk of severe consequences due to invasive fetal blood sampling and IUTs.[4,46] As previously described, the fields has attempted to assess and implement treatments to delay the need for fetal anemia, such as antenatal IVIG with or without plasmapheresis. Yet, recent evidence on the effectiveness of these treatments is scarce. As described above, the multicenter study by Zwiers *et al*. showed a delay in the onset of fetal anemia among pregnancies with a history of early onset severe HDFN treated with IVIG.[19] However, a majority of pregnancies that received IVIG still required IUTs at a relatively low gestational age, with many still requiring multiple IUTs.[19]

In recent years, several pharmaceutical companies have developed inhibitors of the neonatal Fc receptor (FcRn), including nipocalimab[47], rozanolixizumab[48], and efgartigimod[49], to potentially manage alloimmune and autoimmune IgG-mediated diseases.[50] Since FcRn plays a critical role as the sole receptor responsible for IgG recycling and the transplacental transfer of IgG in pregnancy, these FcRn inhibitors hold promise as a novel treatment option for managing HDFN. Nipocalimab, a fully human, aglycosylated, monoclonal IgG antibody was developed by Momenta Pharmaceuticals, later acquired by Johnson & Johnson, and its potential value in treating early onset severe HDFN is being studied.[47] In a completed phase 2 study researchers found that nipocalimab treatment decreased circulating maternal IgG by 85% compared to baseline and was associated with a significantly decreased occurrence of fetal anemia and need for IUTs in 13 pregnancies with a history of early onset severe HDFN underlying its potential value in this at-risk population.[47] However, bearing in mind that nipocalimab inhibits recycling and transplacental transfer of both pathological and beneficial IgG, further data is required on the effects on neonatal immunity and the immune responses of children whose mother was treated with nipocalimab.[51]

Building on the initial publication of the UNITY-trial, in **Chapter 11** we reported on the safety outcomes until 96 weeks after delivery in infants born to maternal participants that received nipocalimab for early onset severe HDFN. We established that fetuses were not exposed to clinically significant nipocalimab exposure in pregnancy, and that neonates were not exposed to clinically relevant nipocalimab concentrations through breastmilk. Even though neonates showed decreased IgG levels at birth, as was expected based on nipocalimab's mechanism of action, IgG levels recovered physiologically, and importantly, we found no unusual or unexpected infections in

the follow-up period. Lastly, although based on limited data, infants showed humoral responses to diphtheria and tetanus vaccinations.

All in all, FcRn blockade is an emerging and promising treatment option in the fetal medicine field. However, its clinical application may be limited to alloimmunized pregnancies with a history of IUTs for fetal anemia. This is because current serological monitoring methods, which are designed to timely detect pregnancies at risk of fetal anemia, primarily facilitate early sonographic monitoring. The widespread use of antibody titers at predetermined critical values is associated with a relatively high rate of false-positive results[2,52], particularly in pregnancies with non-RhD and non-K1 (Kell) alloantibodies.[53,54] Furthermore, there is no consensus on the appropriate critical values to apply, and variations between different laboratories may exist owing to differences in techniques or materials used.[54,55] As a result, relying on the current serological tests and their thresholds to guide nipocalimab treatment may result in overtreatment in a large proportion of alloimmunized pregnancies. Restricting nipocalimab treatment to pregnancies with a history of IUT for HDFN - especially those with early onset severe HDFN, defined as the need for an IUT before 24 weeks of gestation or fetal and neonatal death due to HDFN - will help reduce the rate of overtreatment. This approach is supported by the fact that approximately 86% of pregnancies with an IUT in a previous pregnancy require an IUT in a subsequent pregnancy.[56] Alternatively, the field could explore new serological tests in an attempt to increase specificity, whilst maintaining a 100% sensitivity, as reported in **Chapter 10**.

Yet, further data on the clinical effectiveness and cost-effectiveness of FcRn-inhibitors is required before implementation. Ideally, long-term follow-up of children born to mothers treated with FcRn-inhibitors is essential, ideally extending until at least 8 years of age to thoroughly assess its impact on IgG levels, humoral immune responses and, not to be forgotten, neurodevelopmental outcomes.

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