Optimising neonatal management of haemolytic disease of the foetus and newborn

Ree, I.M.C.

Optimising neonatal management of haemolytic disease of the foetus and newborn

Isabelle Ree

Optimising neonatal management of haemolytic disease of the foetus and newborn

Isabelle Ree

Optimising neonatal management of haemolytic disease of the foetus and newborn

ISBN:	978-94-6458-032-7
Layout and design:	Publiss | www.publiss.nl
Printing:	Ridderprint | www.ridderprint.nl

The printing of this thesis was financially supported by the Department of Paediatrics of the LUMC, Chiesi Pharmaceuticals, Walaeus University Library and Sanquin Blood Supply.

Optimising neonatal management of haemolytic disease of the foetus and newborn

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Leiden,

op gezag van rector magnificus prof. dr. ir. H. Bijl,

volgens besluit van het college voor promoties

te verdedigen op dinsdag 26 april 2022

klokke 11.15 uur

door

Isabelle Mirthe Carmen Ree

geboren te Capelle aan den IJssel

in 1991

Promotores

Prof. dr. E. Lopriore

Prof. dr. M. de Haas

Co-promotor

Dr. J.M.M. van Klink

Leden promotiecommissie

Prof. dr. A.B. te Pas, Leiden Universitair Medisch Centrum, Leiden

Prof. dr. I.K.M. Reiss, Erasmus Medisch Centrum, Rotterdam

Dr. E. Tiblad, Karolinska Institutet, Stockholm

Dr. C.V. Hulzebos, Universitair Medisch Centrum Groningen, Groningen

Aan mijn ouders

TABLE OF CONTENTS

PART ONE	**OVERVIEW**	
	General introduction	11
Chapter 1	Neonatal management and outcome in alloimmune haemolytic disease	21
PART TWO	**PREDICTORS OF SEVERE DISEASE**	
Chapter 2	Foetal bilirubin as predictor of exchange transfusion in severe haemolytic disease of the foetus and newborn	49
Chapter 3	Suppression of compensatory erythropoiesis in haemolytic disease of the foetus and newborn due to intrauterine transfusions	61
PART THREE	**NEONATAL MANAGEMENT AND COMPLICATIONS**	
Chapter 4	Exchange transfusions in severe alloimmune haemolytic disease of the foetus and newborn: a 20-year overview on the incidence, associated risks and outcome	85
Chapter 5	Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth	103
Chapter 6	Randomised controlled trial on the use of EPO to reduce postnatal transfusions in neonates with red blood cell alloimmunisation treated with intrauterine transfusions (protocol)	127
Chapter 7	Necrotising enterocolitis in haemolytic disease of the newborn: a retrospective cohort study	135
PART FOUR	**LONG-TERM OUTCOME**	
Chapter 8	School performance and behavioural functioning in children after intrauterine transfusions for haemolytic disease of the foetus and newborn	149

PART FIVE **SUMMARY AND DISCUSSION**

Summary 166

Nederlandse samenvatting 170

General discussion and future perspectives 175

PART SIX **APPENDICES**

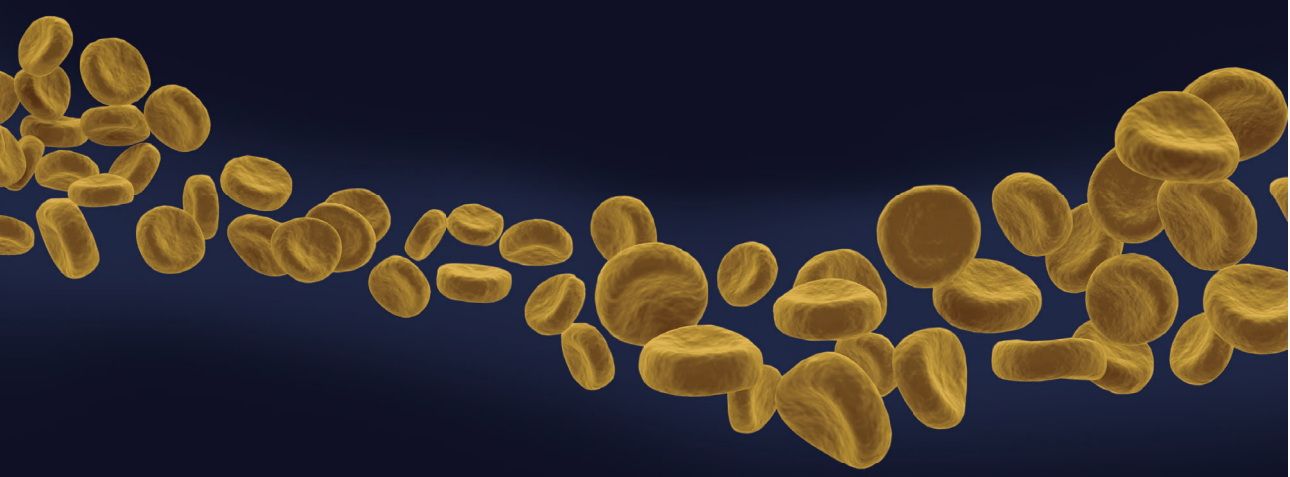
List of abbreviations 190

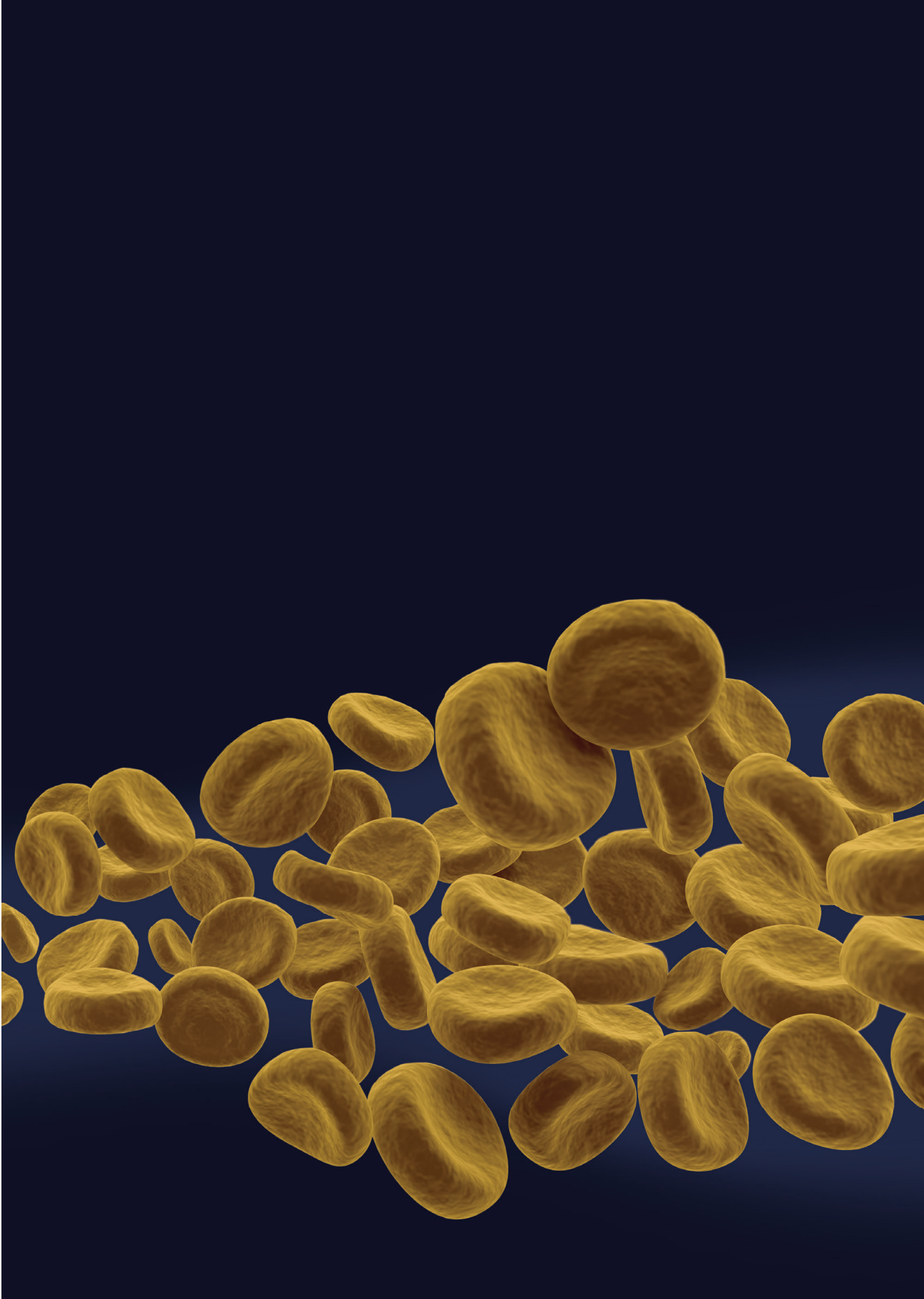
List of publications 191

Curriculum vitae 192

Dankwoord 193

Part 1 - Overview





General Introduction

General introduction

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which the red blood cells of the foetus and the newborn child are destructed due to maternal IgG red blood cell alloantibodies that are transported across the placenta. The process of destruction of foetal red blood cells is named haemolysis.[1,2] The effects of maternal alloimmunisation to foetal red blood cell antigens, range from anaemia and hydrops in the foetus, to hyperbilirubinaemia and kernicterus in the newborn (Figure 1).[2]

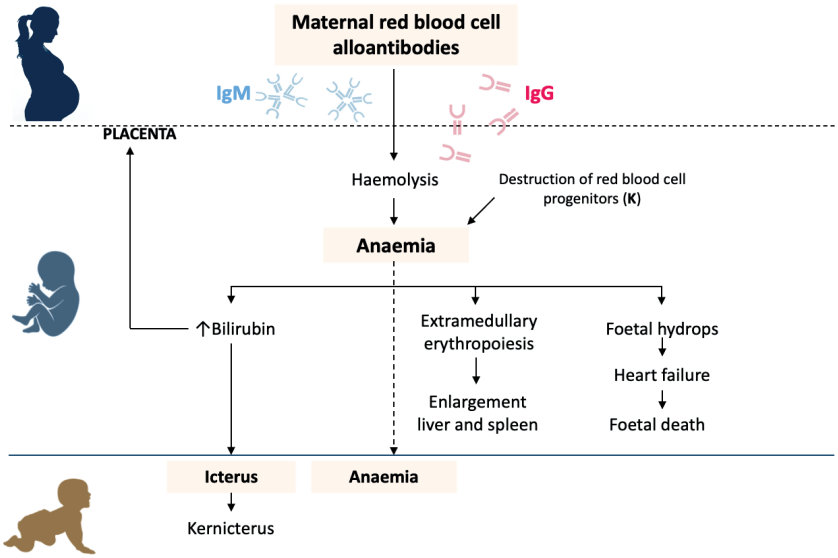


Figure 1. Pathogenesis of haemolytic disease of the foetus and newborn in the mother, foetus and newborn child. *Adapted from De Haas, et al. Vox Sang 2015.*[1]

Historic overview

The symptoms of HDFN were first described by a French midwife named Louise Bourgeois in 1609. She described the birth and death of a twin pair: one baby was very swollen (hydropic) and died shortly after birth, the other, initially in good condition, developed jaundice and neurological symptoms (kernicterus) and died three days later.[3] In 1932, paediatric haematologist Dr. Louis Diamond identified these two conditions, hydrops foetalis and kernicterus, as two aspects of the same condition.[4] The first to hypothesise the antibody-related pathogenesis of HDFN, was pathologist Dr. Ruth Darrow in 1938. Following the loss of Darrow's third child to this disease, she correctly hypothesised that HDFN was caused by

destruction of red blood cells due to an incompatibility of maternal and foetal red blood cells and formation of antibodies in the mother's blood.[5] The nature of these antibodies was further clarified in 1940 by Dr. Karl Landsteiner, Dr. Alexander Wiener and Dr. Philip Levine, as the Rhesus (Rh) blood group system was discovered and the RhD antigen was identified.[6,7]

The most successful step in antenatal management of HDFN was prevention by the introduction of RhD immunoprophylaxis (RhIg) in 1969. Before immunoprophylaxis became available, HDFN affected 1% of all newborn children worldwide and the mortality rate in affected foetuses was up to 50%.[8] Besides prevention, a corner stone of antenatal management is early identification of pregnancies at risk. Since the 1960s, RhD-negative women are routinely screened for the presence of alloantibodies in the last trimester of pregnancy in the Netherlands. Since 1998, all pregnant woman are routinely screened for antibodies in the first trimester, which highly contributed to a drastic decline in the occurrence of hydrops from 39% before 1998, to 15% afterwards.[9]

Monitoring of pregnancies at risk was first done in the 1950s by taking samples of the amniotic fluid and measuring the haemoglobin breakdown products (iron and urobilinogen), the so-called Liley index.[10] From the late 1990s onwards, the non-invasive assessment of the velocity of the blood flow in the middle cerebral artery by Doppler ultrasound has become standard of practice to assess foetal anaemia.[11]

For a long time, induced (preterm) labour of the foetus before it was too severely affected, was the only antenatal treatment option after identification of foetal anaemia. This changed in 1963, when Dr. Albert Liley introduced the procedure of intrauterine blood transfusion (IUT).[12] The complication risk of this procedure was unfortunately extensive after its first introduction, as the first IUTs were X-ray guided transfusions into the foetal peritoneum. Outcomes improved with more experience and the introduction of an ultrasound guided intravascular approach in the 1980s, into the umbilical vein.[13] Another antenatal treatment option is administration of intravenous immunoglobulins (IVIg). IVIg treatment seems to have a beneficial effect to postpone early IUTs in high-risk pregnancies, i.e. pregnancies of alloimmunised women with a history of severe haemolytic disease.[14]

Postnatal care revolves around the treatment of hyperbilirubinaemia by intensive phototherapy and exchange transfusions. Exchange transfusions were first performed for this cause in 1946 by Dr. Harry Wallerstein.[15] Exchange transfusions lower the serum bilirubin level and remove the antibody coated neonatal red blood cell and circulating maternal antibodies to reduce further red blood cell destruction. Approximately 85% of the neonatal blood is replaced by irradiated donor

blood, which has the additional benefit of directly treating anaemia.[16,17] Exchange transfusion is an invasive procedure and although current mortality rates are less than 0.3% in term children, morbidity rates are still high (up to 24%).[18] Phototherapy, as a non-invasive alternative, has rapidly replaced exchange transfusions as standard of care for hyperbilirubinaemia after it was first introduced in 1958. It was discovered after observations of a nurse at a neonatal care unit in England, that children exposed to sunshine showed improvement of their jaundice.[19]

Pathophysiology

Maternal alloimmunisation due to RhD and K (Kell blood group system) antigens is now known as the most common cause of severe HDFN, but varying degrees of alloimmunisation can be triggered by more than 50 different red blood cell antigens.[21] The clinical relevance of alloantibodies in pregnancy depends on the class of antibodies that are produced (only IgG antibodies are transported across the placenta), the presence of the specific antigen against which the alloantibodies are directed on the foetal red blood cells, and the level of expression of the specific antigen on the foetal red blood cells, as some red blood cell antigens are hardly expressed before birth.[22] In general, severe HDFN is most frequently caused by antibodies directed against RhD, K, or Rhc antigens and rarely by antibodies directed against other red blood cell antigens such as RhE, the Jk-antigens of the Kidd antigen system, or Fy-antigens of the Duffy antigen system.[1]

RhD and K-mediated HDFN are known to have a different pathophysiology and clinical course. In K alloimmunisation, even low antibody titres in pregnancy are associated with severe foetal anaemia that usually occurs more early in pregnancy compared to HDFN caused by RhD alloimmunisation. Overall, K alloimmunisation accounts for a higher need for IUTs and in general more IUTs per pregnancy.[23] K antigens appear on erythroid progenitor cells early in erythropoiesis,[24] and erythroid suppression seems to be the predominant mechanism in producing foetal anaemia, rather than haemolysis.[25-27] Although children affected by K alloimmunisation require overall less phototherapy and less exchange transfusions compared to RhD alloimmunisation, there is in both RhD and K alloimmunisation a similarly high degree of neonatal anaemia and transfusion dependency after birth.[28]

Aim and outline of this thesis

Prevention and therapy of HDFN is generally considered a success story in perinatal medicine. In a relatively short time span, the pathophysiology of HDFN was unravelled, effective prevention programs were implemented worldwide, and successful antenatal and postnatal treatment strategies in experienced centres further minimalised the risk of morbidity and mortality. Nonetheless, various aspects remain to be elucidated and there is still room to optimise both the antenatal and postnatal management and improve the short and long-term outcomes of this disease.

This thesis aims to add to the knowledge on the use and outcome of current therapy options in HDFN. An overview of the current neonatal management of HDFN is provided and the current standard procedures as exchange transfusions and top-up red blood cell transfusions and associated complications are thoroughly evaluated, as well as long-term outcomes after intrauterine transfusions (IUTs). This thesis also aims to set a base for further individualisation of treatment, in which a better selection of high-risk and low-risk cases should lead to a minimisation of late interventions. With this knowledge, this thesis strives to further contribute to successful management of HDFN.

Outline

PART ONE	**OVERVIEW**
	General introduction
Chapter 1	Review on the available literature on neonatal management and outcome in HDFN.
PART TWO	**PREDICTORS OF SEVERE DISEASE**
Chapter 2	Cohort study examining the use of foetal bilirubin as predictor of exchange transfusion.
Chapter 3	Cohort study on the suppressive effect of intrauterine transfusions on compensatory erythropoiesis.
PART THREE	**NEONATAL MANAGEMENT AND COMPLICATIONS**
Chapter 4	Cohort study evaluating the incidence, associated risks and outcome of exchange transfusions over the last 20 years.
Chapter 5	Cohort study on the prediction of anaemia and transfusion dependency in the first three months after birth.
Chapter 6	Randomised controlled trial on the use of EPO to reduce postnatal transfusions in neonates with red blood cell alloimmunisation treated with intrauterine transfusions (protocol).
Chapter 7	Cohort study examining the association of necrotising enterocolitis with haemolytic disease of the newborn.
PART FOUR	**LONG-TERM OUTCOME**
Chapter 8	Questionnaire cohort study evaluating school performance and behavioural functioning in children after intrauterine transfusions.
PART FIVE	**SUMMARY AND DISCUSSION**
	Summary Nederlandse samenvatting General discussion and future perspectives

References

1. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. Vox Sang 2015;109:99-113.
2. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev 2000;14:44-61.
3. Bourgeois L. Observations diverses sur la sterilité, perte de fruict, foecondité, accouchements et maladies des femmes et enfants nouveaux naiz. Paris; 1609
4. Diamond LK, Blackfan KD, Baty JM. Erythroblastosis fetalis and its association with universal edema of the fetus, icterus gravis neonatorum and anemia of the newborn. J Pediatr 1932;1:269-276.
5. Darrow RR. Icterus gravis (erythroblastosis neonatorum), examination of etiologic considerations. Arch Pathol 1938;25:378-417.
6. Landsteiner K, Wiener AS. An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc Soc Exp Biol Med 1940;42:223-232.
7. Levine P, Burnham L, Katzin EM, Vogel P. The role of iso-immunization in the pathogenesis of erythroblastosis fetalis. Am J Obstet Gynecol 1941;42:925-937.
8. Bowman J. Thirty-five years of Rh prophylaxis. Transfusion 2003;43:1661-1666.
9. Zwiers C, Oepkes D, Lopriore E, Klumper FJ, de Haas M, van Kamp IL. The near disappearance of fetal hydrops in relation to current state-of-the-art management of red cell alloimmunization. Prenat Diagn 2018;38(12):943-950.
10. Bevis DCA. Composition of liquor amnii in haemolytic disease of the newborn. Lancet 1950;2:443.
11. Oepkes D, Seaward PG, Vandenbussche F, Windrim R, Kingdom J, Beyene J, Kanhai HHH, et al. Doppler ultrasonography versus amniocentesis to predict fetal anemia. N Engl J Med 2006;355:156-62.
12. Liley AW. Intrauterine transfusion of fetus in haemolytic disease. Br Med J 1963;2:1107-1108.
13. Berkowitz RL, Hobbins JC. Intrauterine transfusion utilizing ultrasound. Obstet Gynecol 1981;57:33.36.
14. Zwiers C, Van der Bom JG, Van Kamp IL, Van Geloven N, Lopriore E, Smoleniec J, Devlieger R, et al. Postponing Early intrauterine Transfusion with Intravenous immunoglobulin Treatment; the PETIT study on severe hemolytic disease of the fetus and newborn. Am J Obstet Gynecol 2018;219(3):291.e1-291.e9.
15. Wallerstein H. Treatment of severe erythroblastosis by simultaneous removal and replacement of blood of the newborn. Science 1946;103:583-584.
16. Dennery PA, Seidman DS, Stevenson DK. Neonatal hyperbilirubinemia. N Engl J Med 2001;344:581-890.
17. Murki S, Kumar P. Blood exchange transfusion for infants with severe neonatal hyperbilirubinemia. Semin Perinatol 2011;35:175-184.
18. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal exchange transfusions and its effect on exchange-related morbidity and mortality. Pediatrics 2007;120:27-32.
19. Cremer RJ, Perryman PW, Richards DH. Influence of light on the hyperbilirubinaemia of infants. Lancet 1958;1:1094-1097.
20. Stokowski LA. Fundamentals of phototherapy for neonatal jaundice. Adv Neonatal Care 2011;11:S10-21.
21. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med 2008;13:207-214.

22. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de Haas M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. Transfusion 2008;48(5):941-952.

23. Slootweg YM, Lindenburg IT, Koelewijn JM, Van Kamp IL, Oepkes D, De Haas M. Predicting anti-Kell-mediated hemolytic disease of the fetus and newborn: diagnostic accuracy of laboratory management. Am J Obstet Gynecol 2018;219(4):393 e391-393 e398.

24. Southcott MJ, Tanner MJ, Anstee DJ. The expression of human blood group antigens during erythropoiesis in a cell culture system. Blood 1999;93(12):4425-4435.

25. Daniels G, Hadley A, Green CA. Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion 2003;43(1):115-116.

26. Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anemia. N Engl J Med 1998;338(12):798-803.

27. Vaughan JI, Warwick R, Letsky E, Nicolini U, Rodeck CH, Fisk NM. Erythropoietic suppression in fetal anemia because of Kell alloimmunization. Am J Obstet Gynecol 1994;171(1):247-252.

28. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, van Kamp IL, Oepkes D, Walther FJ, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100(3):312-316.

Chapter one

Neonatal management and outcome in alloimmune haemolytic disease

Isabelle M.C. Ree
Vivianne E.H.J. Wintjens
Anske (J.)G. van der Bom
Jeanine M.M. van Klink
Dick Oepkes
Enrico Lopriore

Expert Review of Hematology 2017;10(7):607-616.

Abstract

Introduction

Haemolytic disease of the foetus and newborn (HDFN) occurs when foetal and neonatal erythroid cells are destroyed by maternal erythrocyte alloantibodies, it leads to anaemia and hydrops in the foetus, and hyperbilirubinaemia and kernicterus in the newborn. Postnatal care consists of intensive phototherapy and exchange transfusions to treat severe hyperbilirubinaemia and top-up transfusions to treat early and late anaemia. Other postnatal complications have been reported such as thrombocytopenia, iron overload and cholestasis requiring specific management.

Areas covered

This review focusses on the current neonatal management and outcome of haemolytic disease and discusses postnatal treatment options as well as literature on long-term neurodevelopmental outcome.

Expert commentary

Despite major advances in neonatal management, multiple issues have to be addressed to optimise postnatal management and completely eradicate kernicterus. Except for strict adherence to guidelines, improvement could be achieved by clarifying the epidemiology and pathophysiology of HDFN. Several pharmacotherapeutic agents should be further researched as alternative treatment options in hyperbilirubinaemia, including immunoglobulins, albumin, phenobarbital, metalloporphyrins, zinc, clofibrate and prebiotics. Larger trials are warranted to evaluate erythropoietin, folate and vitamin E in neonates. Long-term follow-up studies are needed in HDFN, especially on thrombocytopenia, iron overload and cholestasis.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which foetal and neonatal erythroid cells are destroyed by maternal erythrocyte IgG alloantibodies that are transported across the placenta.[1,2] Maternal alloimmunisation can be triggered by more than 50 different erythrocyte antigens, but most severe cases involve the Rhesus D antigen (RhD) and cause Rh-mediated haemolytic disease.[3] The effects of the incompatibility of maternal and foetal erythrocytes range from anaemia and hydrops in the foetus, to hyperbilirubinaemia and kernicterus in the newborn.[2] Management of HDFN is therefore a two-way effort, concerning both antenatal as well as postnatal strategies. The most successful step in antenatal care of HDFN was the introduction of RhD immunoprophylaxis in 1968.[4] Before immunoprophylaxis became available, HDFN affected 1% of all newborns worldwide and the mortality rate in affected foetuses was up to 50%.[4] The incidence of Rh haemolytic disease is now approximately 0.5% in RhD-negative women in industrialised countries.[2,4] However, despite adequate RhD immunoprophylaxis, 1 to 3 in 1000 RhD negative women still develop anti-D antibodies.[5] Antenatal care not only involves immunoprophylaxis, but also the monitoring of high-risk cases by laboratory testing and ultrasound-based techniques in order to detect foetal anaemia. If necessary, severe foetal anaemia is treated with intrauterine blood transfusions (IUTs) to prevent or treat foetal hydrops.[6,7] At least equally important in the management of HDFN is postnatal care. Postnatal care involves the management and stabilisation of the ill and frequently premature newborn[8], but revolves around the treatment of hyperbilirubinaemia and prevention of kernicterus by intensive phototherapy and exchange transfusions. Except for hyperbilirubinaemia, other postnatal complications of HDFN and their respective treatments have been reported and studied such as early and late anaemia. To date, however, little attention has been devoted to complications such as thrombocytopenia, iron overload and cholestasis. The long-term neurodevelopmental outcome has only recently been investigated in large cohorts. With the ongoing research on HDFN, it is important to highlight and summarise recent developments and to point out the remaining challenges for the future. This review focuses on the current postnatal management and outcome of HDFN. Recent literature is reviewed and discussed in a clinical point of view to assess current and future care of these neonates. The main issues addressed in this paper are the various postnatal treatment options of hyperbilirubinaemia and anaemia, as well as long-term outcome.

Management of hyperbilirubinaemia

In severe hyperbilirubinaemia, unconjugated bilirubin can cross the blood-brain barrier and can potentially lead to bilirubin neurotoxicity. A condition defined as bilirubin-induced neurologic dysfunction (BIND) occurs, of which kernicterus or chronic bilirubin encephalopathy is known as its most severe and permanent clinical manifestation.[9] In the acute phase of BIND, symptoms include lethargy, hypotonia and feeding problems. Untreated, hyperbilirubinaemia can cause fever, a high-pitched cry and hypertonia, eventually leading to apnoea, coma, seizures and death. In kernicterus, the most affected brain tissues are the basal ganglia, cerebellum and various brainstem nuclei. Long term morbidity in surviving infants include athetoid cerebral palsy, hearing loss and intellectual disability.[10,11]

Standard care of hyperbilirubinaemia consists of intensive phototherapy and, if indicated, exchange transfusions. Improvement of these treatments remains a constant and recurring focus of research, as well as the search for alternative treatments such as pharmacotherapeutic agents that might enhance treatment of hyperbilirubinaemia in HDFN.

Intensive phototherapy

Since its introduction in the 1970s, phototherapy has been the main treatment for neonatal hyperbilirubinaemia. Phototherapy causes photo isomerisation of bilirubin in the skin to water-soluble isomers that can be excreted by the kidneys and stool without further metabolism by the liver. The efficacy of phototherapy depends on several factors, including the wavelength of the light used, the intensity of the light source, the total light dose (time under phototherapy and amount of skin exposed), and/or the threshold at which phototherapy is commenced.[12-15] The light used in phototherapy needs to penetrate the skin and be absorbed by bilirubin, which happens with blue or green light with an optimal wavelength range of 460-490 nm.[12] The intensity or irradiance (energy output) of the light source is measured with a radiometer in units of watts per square centimetre or in microwatts per square centimetre per nanometre over a given wavelength band.[12] Light emitting diodes (LEDs) are the most commonly used phototherapy lamps, but also halogen lamps and fiberoptic systems such as biliblankets are used.[10] A recent systematic review by Woodgate et al. recommends close phototherapy (distance of 20 cm above the neonate) over distant light-source phototherapy (40 cm above the neonate), and double over single phototherapy, thus increasing the light irradiance. No clear benefit was found for continuous over intermittent phototherapy in neonates more than 2000 g or term infants, or for higher skin exposure by removing the diaper. Phototherapy should be applied once serum bilirubin levels reach predefined thresholds.[15]

In HDFN, the use of intensive phototherapy is recommended. Intensive phototherapy defined by the American Academy of Pediatrics (AAP) as a spectral irradiance of at least 30 µW per square centimetre per nanometre over the same bandwidth delivered to as much of the body-surface area as possible. In practice, intensive phototherapy is continuous with at least two lamps closely above the neonate and a biliblanket covering the whole body and back side of the neonate.[10]

Phototherapy is generally considered a safe procedure and reports of significant toxicity are exceptionally rare and mostly limited to case reports.[10,16] Recently, two studies were published on a possible link between phototherapy and development of cancer. Specifically, the risk for myeloid leukaemia and kidney cancer seemed slightly increased. However, after correcting for congenital and chromosomal abnormalities, the associations were no longer statistically significant.[17,18] Additionally, previous studies could not find an association between phototherapy and skin cancer.[16,19] Phototherapy does seem to be associated with the development of allergic diseases.[20]

Fluid supplementation/hydration

Fluid supplementation during phototherapy may help the excretion of photoproducts through both urine and bile and to overcome the increased insensible water loss.[21] However, with the increased use of LED lights, the beneficial effect of fluid supplementation in neonates without dehydration becomes questionable, as LED lights have a much lower heat output and therefore less insensible water loss.[12,14] Intravenous fluid support has no effect on the rate of decrease in serum bilirubin and decrease in duration of phototherapy in healthy term newborns with no dehydration.[22,23]

Exchange transfusion

In the 1940s, treatment of neonatal hyperbilirubinaemia with exchange transfusions was introduced, initially as specific treatment for Rh haemolytic disease.[24] Exchange transfusions lower the serum bilirubin level and remove the antibody-coated neonatal erythrocytes and circulating maternal antibodies to reduce further erythrocyte destruction. Approximately 85% of the neonatal blood is replaced by irradiated donor blood, which has the additional benefit of providing new albumin with bilirubin binding sites and directly treating anaemia.[25,26] However, exchange transfusions are not without risk. Current mortality rates are less than 0.3% in term neonates, but increases above 10% in preterm neonates.[27,28] Morbidity rates are still high (up to 24%), even in term neonates and include cardio-respiratory instability, apnoea, catheter-related complications, thrombocytopenia and infections.[27-29]

There is no clear benefit for the use of single volume exchange transfusion as opposed to double volume exchange transfusion; double volume exchange transfusions is current practice.[30] Two-stage exchange transfusions showed to be more efficient than one-stage exchange transfusions in term neonates in single-volume transfusions. In the two-stage procedure, a first exchange transfusion is given with a three hour resting period (allowing for equilibrium of bilirubin to be established between the intravascular and extravascular compartments), followed by a second stage. Two-stage exchange transfusion significantly lowered rebound serum bilirubin and the need for repeated exchange transfusions. There was no difference in mortality and morbidity between the two groups.[31]

The frequency of exchange transfusions sharply declined since its introduction due to the emergence of phototherapy, the development of RhD immunoprophylaxis and improved monitoring, and care for hyperbilirubinaemia.[28] Exchange transfusions are now recommended if bilirubin levels remain above exchange transfusion thresholds despite intensive phototherapy, or if signs of acute bilirubin encephalopathy occur.[10] The rate of exchange transfusion in HDFN decreased to around 20% after these more restrictive guidelines were published by the AAP.[10,32]

In our centre, the exchange transfusion rate in HDFN dropped from approximately 70% before 2005[33] to approximately 15% thereafter.[32] This reduction was due to the implementation of a more restrictive guideline for exchange transfusions in terms of bilirubin cut-off values, especially by introducing cut-off values for different risk groups, compared to the more liberal guideline used at our centre before 2005.

Intravenous immunoglobulin

Some studies have shown a reduced need for exchange transfusions in HDFN with the use of intravenous immunoglobulins.[34] Immunoglobulins seem to block Fc receptors on macrophages, which reduces the breakdown of antibody coated erythrocytes and lowers the circulating unconjugated bilirubin levels.[35] Administration of intravenous immunoglobulin (0.5-1 g/kg) is currently recommended by the AAP if the total serum bilirubin is rising despite intensive phototherapy or the bilirubin level is within 2 to 3 mg/dL (34-51 μmol/L) of the exchange level. If necessary, a repeated dose can be given in 12 hours.[10,34]

Despite current recommendations, a recent randomised controlled trial by Santos et al. and a recent randomised controlled trial in our centre by Smits-Wintjens et al., could not support the efficacy of intravenous immunoglobulins in HDFN.[32,36] As efficacy is questioned, it is important to point out that although intravenous immunoglobulins are considered a safe

treatment, rare but serious side effects have been reported, including transfusion transmitted diseases, anaphylaxis, hypersensitivity, thrombosis, pulmonary emboli and renal failure.[37] A possible association with necrotising enterocolitis (NEC) was found in a retrospective study comparing near-term infants with Rh haemolytic disease treated with immunoglobulins with a control group without immunoglobulin treatment.[38] In a meta-analysis by Yang et al., immunoglobulins showed an increased risk for NEC (odds ratio 4.53; 95%-confidence interval 2.34-8.79; $p<.001$), but not for final mortality.[39] A recent systematic review advocates further well designed studies before conclusive advice can be given about the treatment of HDFN with intravenous immunoglobulins.[40]

Alternatives

Several pharmacotherapeutic agents have been studied as possible treatment options in neonatal hyperbilirubinaemia. The main focus is on albumin, phenobarbital, metalloporphyrins, zinc, clofibrate and prebiotics. None of these agents is currently recommended as standard care in hyperbilirubinaemia.[10]

Albumin

As albumin binds bilirubin, it is thought that extra administered albumin can lower serum bilirubin levels. However, sufficiently powered trials on the clinical benefits are lacking and albumin administration is not recommended as standard care at this moment.[10] A small randomised controlled trial in India by Shahian et al. showed evidence that albumin administration prior to exchange transfusion is beneficial to lower total serum bilirubin levels post-exchange and lower the mean duration of phototherapy.[41] A subsequent randomised controlled trial did not show the same effects.[42] The AAP marks it as an option to measure the serum albumin level in neonates with hyperbilirubinaemia and to consider an albumin level below 3.0 g/dL as an extra risk factor to lower the phototherapy threshold. Furthermore, if an exchange transfusion is being considered, the serum albumin level should always be measured and with the bilirubin/albumin ratio used in determining the need for exchange transfusions. Albumin supplementation is not recommended.[10]

Phenobarbital

Phenobarbital was used in an attempt to increase biliary flow in the 1970s. Phenobarbital increases hepatic uridine diphosphate (UDP)-glucuronosyl transferase (UGT) activity and the conjugation of bilirubin, and has a possible positive effect on hepatic uptake of bilirubin.[43,44] A recent randomised clinical trial by Kaabneh et al. in neonates with HDFN showed a minor

advantage of adding phenobarbital to phototherapy treatment.[45] However, phenobarbital acts slowly and is therefore not the treatment of choice when adequate phototherapy and exchange transfusions are available. There might be a role for antenatal maternal administration of phenobarbital, as positive results (decreased need for exchange transfusions) were found in a retrospective study by Trevett et al., although these results are yet to be confirmed.[46]

Metalloporphyrins

Metalloporphyrins inhibit heme-oxygenase, the rate-limiting enzyme in the catabolism of haem to bilirubin. In this manner, metalloporphyrins decreases the production of bilirubin, rather than increasing the excretion of bilirubin.[47] Treatment with metalloporphyrins may reduce neonatal bilirubin levels and decrease the need for phototherapy and hospitalisation. Sufficient evidence is lacking however to recommend routine treatment of hyperbilirubinaemia with metalloporphyrins.[48]

Zinc

Oral zinc is thought to reduce the serum bilirubin by decreasing the enterohepatic circulation of bilirubin.[49] However, recent systematic reviews by Mishra et al. and Sharma et al. concluded that there is currently no role for zinc in the treatment of neonatal hyperbilirubinaemia. There seems to be no effect on duration of phototherapy, incidence of phototherapy, or age of starting of phototherapy.[50,51]

Clofibrate

Clofibrate induces glucuronosyl transferase that changes bilirubin in a water-soluble form that can be excreted. It seems that clofibrate in combination with phototherapy lowers the level of bilirubin and shortens the duration of phototherapy in term neonates[52,53], but larger trials are needed to evaluate its effect. Further research is especially needed in haemolytic-caused hyperbilirubinaemia, as these neonates were excluded in some of the trials.[54]

Prebiotic supplementation

Recently, the role of prebiotic supplementation was focus for research on the treatment of neonatal hyperbilirubinaemia. Prebiotics are thought to increase gastrointestinal motility and stool frequency, decrease viscosity of stool and the enterohepatic circulation of bilirubin, and improve feeding tolerance and growth of beneficial bacteria in the gut. Results seem promising in both preterm and term neonates with hyperbilirubinaemia on total serum bilirubin levels as well as duration of phototherapy.[55,56]

Antenatal corticosteroids

Antenatal treatment with corticosteroids has been shown to accelerate lung maturation in preterm infants and reduce the risks of respiratory disorders, especially in those born before 34 weeks' gestation.[57] Although antenatal betamethasone given after 34 weeks' gestation does not appear to decrease the risk of respiratory disorders, recent studies reveal that betamethasone reduces the risk of neonatal jaundice requiring phototherapy, possibly because of acceleration of liver maturation.[58]

Management of anaemia

Haemolysis of the foetal erythrocytes by maternal alloantibodies causes anaemia. These alloantibodies remain in the neonatal circulation after birth for several months and can cause prolonged anaemia. Anaemia in haemolytic disease is divided in early anaemia (onset at birth up to 7 days of age) and late anaemia. Late anaemia occurs in 83% of neonates of a gestational age of 35 weeks or more with HDFN and is further split into "late hyporegenerative anaemia" and "late anaemia of haemolytic disease".[59] Late hyporegenerative anaemia is caused by depressed erythropoiesis and is characterised subsequently by low reticulocyte counts. Underlying mechanisms are proposed to be the following: intramedullary destruction of erythrocyte precursors, bone marrow suppression from intrauterine and postnatal transfusions, erythropoietin (EPO) deficiency, shortened half-life of transfused erythrocytes and relative anaemia due to the expanding intravascular volume of the growing neonate. Late anaemia of haemolytic disease is characterised by age-appropriate or elevated reticulocyte counts, reflecting an active bone marrow to compensate for the shortened erythrocyte survival. This form of late anaemia is thought to be due to a combination of continuing haemolysis by remaining antibodies, shortened survival of transfused erythrocytes, natural decline of the haemoglobin level and the expanding intravascular volume of the growing neonate.[59,60] Interestingly, although late anaemia was already described before IUTs were available, IUTs seem to actually increase the risk of postnatal anaemia. The pathophysiological mechanism is not entirely understood, but treatment with repeated IUTs possibly lead to persistent anaemia due to suppression of erythropoiesis.[33]

Primary treatment of anaemia in HDFN is administering erythrocyte transfusions, known as top-up transfusions. In addition, several pharmacological agents are used to stimulate erythropoiesis.

Top-up transfusions

Late anaemia usually resolves by the third month of life; until then erythrocyte transfusions may be necessary to treat postpartum anaemia.[59,61] The mean postnatal age for a first top-up transfusion in HDFN is 18 days (range 1-34).[62] At least one top-up transfusion is reported to be required in 68 to 83% of neonates with HDFN.[33,59,63] Some neonates need up to 6 top-up transfusions in the first three months of life.[62] In neonates that were treated with IUTs, the top-up transfusion rate is significantly higher (77-89%), than in neonates that were not treated with IUTs (27-67%).[33,59,63] In the IUT group, the median number of top-up transfusions was 1 (range 0-4), compared to 0 (range 0-2) in non-IUT group ($p<.001$).[33] This difference can be explained by a depressed erythropoiesis and is correlated with a significantly lower reticulocyte count at birth in transfused neonates.[33,62,64] Variations in the percentage of neonates requiring top-up transfusions may be explained by various factors, including differences in transfusion guidelines and thresholds for erythrocyte transfusions. In our centre, a top-up transfusion of 15 ml/kg irradiated erythrocytes is indicated in term neonates with HDFN when haemoglobin levels fall below 7.2 g/dL (4.5 mmol/L) or below 8.8 g/dL (5.5 mmol/L) when clinical symptoms of anaemia are present (increased oxygen need, poor feeding, tachycardia and/or tachypnoea).

Exchange transfusion

Exchange transfusions not only have a positive effect on the clearance of bilirubin in haemolytic disease, but as mentioned before, it also treats anaemia as the neonatal blood is replaced by immunologically compatible donor blood and decreases plasma ferritin and iron levels.[25] The reduced need for exchange transfusion in the last decades seems to be associated with an increased need for top-up transfusions. This is probably explained by longer lasting haemolysis due to a reduced clearance of maternal antibodies.[33,63]

Delayed cord clamping

Delayed cord clamping at birth allows additional placental blood transfusion. In a term newborn this transfusion can consist up to one-quarter to one-third of the total neonatal blood volume.[65] In anaemia secondary to erythrocyte alloimmunisation, a significant increase in haemoglobin levels at birth was observed, as well as a longer delay between birth and first transfusion, and a decrease in the number of postnatal exchange transfusions, with no notable adverse effects.[66,67]

Pharmacotherapeutic options

In erythropoiesis, sufficient amounts of EPO, folate, iron and vitamin E are essential. To enhance erythropoiesis, administration of these nutrients may have a beneficial effect in the treatment of anaemia in HDFN.[68,69]

EPO

EPO is the principle growth factor responsible for foetal and neonatal erythropoiesis and is primarily developed in the foetal liver.[70] Since over a decade, recombinant human EPO has been applied in small studies and casuistic reports, with different outcomes for the occurrence of anaemia and the need for top-up transfusions in neonates with HDFN.[71-74] Due to limited clinical importance of observed beneficial effects, routine use of EPO is currently not recommended.[75] A well-designed randomised controlled clinical trial of sufficient sample size is required to establish the role of EPO in the treatment of HDFN[69] and will be carried out by our centre in the near future. In this trial, included neonates will be randomised at birth to treatment with darbepoetin alfa (Aranesp®) or conventional care. The intervention group will receive darbepoetin alfa subcutaneously once a week in 10 U/kg/dose for 8 weeks.

Folate

Folate plays a role in the proliferation of erythroblasts during their differentiation.[68] Very limited data is available on its therapeutic efficacy, although a small effect has been seen in the addition of both vitamin B12 (3 microg/kg/day) and folate (100 microg/kg/day) to EPO as treatment in anaemia of prematurity.[76] Due to the prolonged anaemia often seen in HDFN, folate is supplemented in our centre from 3 months postpartum in a dosage of 250 µg/day.

Iron

Iron is required for haemoglobin synthesis by erythroblasts. The use of iron in anaemia associated with HDFN has been studied most extensively in combination with EPO. Administration of EPO without, or with, low iron supplementation can lead to iron deficiency and ineffective erythropoiesis.[69,77] However, neonates with HDFN tend to have an iron overload due to the combination of prolonged haemolysis and treatment with multiple IUTs and erythrocyte transfusions. Based on current knowledge, iron supplementation should be withheld, especially in transfused infants as it is associated with numerous potential adverse effects.[78,79]

Vitamin E

It is unclear whether vitamin E acts as an antioxidant that protects the erythrocyte membrane or as an erythropoietic factor, but shortage of vitamin E might be an additional cause of anaemia.[80] Limited data are available on the therapeutic effects of vitamin E. A pilot study of Pathak et al. showed that oral supplementation at 50 IU/day does not enhance the response of preterm neonates to erythropoietin and iron, compared to placebo.[81]

Management of other complications

BIND and kernicterus

Although rare, BIND and kernicterus are still occurring in the industrialised world. According to rough estimates based on national registration systems, a severely high bilirubin count (>420 µmol/l) occurs in 100-200 infants a year in the Netherlands (i.e. 0.5-1 per 100 live births). Although cases of kernicterus are reported several times a year, precise estimates on the prevalence of kernicterus are not available.[82] Alarmingly, throughout the 1990s, a significant rise in case reports of kernicterus occurred compared to previous years. This rise was attributed to a combination of reduced concern for jaundice in newborns, early discharge with inadequate follow-up and a decreased awareness of the long-term complications of hyperbilirubinaemia. Guidelines were then updated and implemented accordingly.[83] Cornerstone of the management of BIND and kernicterus is timely and adequate treatment of hyperbilirubinaemia. Rather than applying phototherapy and exchange transfusions when necessary, the treatment of hyperbilirubinaemia is a complex interplay of adequate screening of at-risk pregnancies, anticipation of the hyperbilirubinaemic neonate, and the combination of antenatal and postnatal management. The importance of this interplay is also reflected in the nonlinear relationship between neonatal bilirubin levels and the risk of BIND and kernicterus and the wide variety of clinical manifestations, varying between neurodevelopmental difficulties (including developmental delay, cognitive impairment, and disordered executive function), and behavioural and psychiatric disorders.[84]

Thrombocytopenia

In severe HDFN, thrombocytopenia is believed to be caused by increased erythropoiesis as it can suppress the other cell lines and potentially cause leukopenia and thrombocytopenia.[85] A retrospective study in our centre showed a 26% incidence of thrombocytopenia (platelet

count <150·10^9/L) in neonates treated with IUT for RhD haemolytic disease. The occurrence of thrombocytopenia was an independent risk factor for perinatal mortality.[86] A more recent retrospective observational study was carried out in our centre and also showed a similar incidence of 26% of thrombocytopenia in all neonates with HDFN at birth. Severe thrombocytopenia was found in 6% of neonates. Three risk factors were independently associated with thrombocytopenia at birth: treatment with IUT, being born 'small-for-gestational-age' (defined as a birth weight below the 10th percentile) and lower gestational age at birth.[87] Thrombocytopenia in HDFN is usually a self-limiting disease, but treatment with platelet transfusions may be needed if platelets drop below transfusion-threshold.

Iron overload

As mentioned before, neonates with Rh haemolytic disease tend to have an iron overload. This is caused by the combination of prolonged haemolysis and treatment with multiple IUTs and erythrocyte transfusions.[72,73,88] Although iron is essential in early brain development and function, iron overload can have detrimental health effects as well. It causes damage to the liver, heart, and endocrine organs, alters immune response and increases susceptibility to infection.[89] Iron deficiency in the first 3 months of life is very rare in HDFN and has not been described in neonates that received postnatal top-up transfusions. Iron overload occurs in 70% of neonates with HDFN at birth, 50% at the age of 1 month and 18% at the age of 3 months. In neonates with HDFN that received IUTs, ferritin levels were twice as high compared to controls at birth (mean 598 ± 249 µg/l vs 270 ± 111 µg/l, p<.001).[73] The use of iron supplementation in the first 3 months of life in neonates with severe HDFN is strongly discouraged, particularly after IUTs.

Cholestasis

Conjugated hyperbilirubinaemia (cholestasis) has been described in neonates with HDFN[90,91] and can give rise to suspicion of underlying liver disease with additional diagnostic measures. A retrospective study in our centre showed a total incidence of cholestasis in infants with RhD-mediated HDFN of 13% (41/313). Cholestasis in haemolysis might be caused by obstruction of the excretory system by excessive conjugated bilirubin and should primarily be regarded as complication of HDFN. Treatment with one or more IUTs and RhD alloimmunisation were independent risk factors for cholestasis. Cholestasis resolved spontaneously within one week to three months after birth in almost half of the patients, but the authors advocate larger studies to confirm these findings and to determine the exact course and aetiology of cholestasis in HDFN.[92]

Long-term outcome

With the improvements in antenatal and neonatal management over the past decades, survival has significantly improved in HDFN. As mortality rates lower, attention is shifting towards the long-term outcome in the surviving neonates. Adverse prenatal conditions including impaired foetal nutrition and growth can have a profound and long-lasting impact on physical and mental health throughout life, for example increased risk of cardiovascular disease, neuropsychological disorders and metabolic disease.[93,94] The impact of severe foetal and neonatal anaemia, hyperbilirubinaemia and of the (invasive) procedures and treatments foetuses and neonates with HDFN are exposed to is unknown, but seems to justify long-term follow-up studies as current data is limited.[95]

Long-term outcome after IUT

A large long-term follow-up study in our centre, the LOTUS study (Long-Term follow-up after intra-Uterine transfusionS), was performed to determine the incidence of neurodevelopmental impairment (NDI) in children treated with IUT for HDFN (between 1988 and 2008). A total of 291 children were studied at a median age of 8.2 years. NDI was defined as the presence of one or more of the following: cerebral palsy, severe developmental delay, bilateral blindness, and/or deafness. The incidence of severe NDI was 3.1%, comparable to the Dutch normative population (2.3%). The overall rate of NDI was 4.8% (14/291). In a multivariate regression analysis, the number of IUTs, severe neonatal morbidity, parental education and severe prenatal hydrops were independent risk factors associated with NDI.[96]

Health-related quality of life and behavioural functioning in 285 children and adolescents treated with IUT for alloimmune anaemia was recently assessed at our centre.[97] Parents reported lower scores on cognitive functioning in their children aged 6-11 years compared to Dutch norms. Behavioural difficulties were more prevalent than norms and associated with maternal educational level. Overall, in the majority of survivors, long-term outcomes following IUT for alloimmune anaemia appear favourable.

Hypothetically, the chronic anaemia caused by immune haemolysis in the foetus results in the need for increased cardiac output and may lead to myocardial hypertrophy. Limited data show that HDFN treated with IUT may lead to less myocardial mass in childhood, but the long-term cardiovascular consequences in adult life are unknown.[95] However, very recently, a retrospective cohort study by Wallace et al. was published on the cardiac function and cardiovascular risk factors in adults exposed to foetal anaemia and intrauterine transfusion

(n=95), compared to their healthy siblings. The former patients showed smaller left ventricular volumes, increased left ventricular wall thickness, and decreased myocardial perfusion at rest, concluding that cardiovascular development is altered after exposure to foetal anaemia and IUT. It is unknown whether this will have clinical consequences regarding the development of cardiovascular disease as the average age at the time of study was 34 years.[98]

Long-term outcome after immunoglobulins

In a follow-up study of a randomised controlled trial conducted at our centre, we found that the neurodevelopmental outcome in children treated with intravenous immunoglobulins for HDFN was not different from children treated with placebo.[32,99] At a median age of 4 years, NDI was detected in 3% in both the immunoglobulin (1/34) and placebo (1/32) group. After stratification for treatment with or without IUT, similar results were obtained. Standardised long-term follow-up studies with large enough case series and sufficient power are needed to replicate these findings.

Conclusion

Over the years, neonatal management and outcome in HDFN improved significantly. Classical therapies such as intensive phototherapy and exchange transfusions for hyperbilirubinaemia and erythrocyte transfusions for anaemia are well studied, but are still improved upon. Alternative treatments have been suggested such as albumin, phenobarbital, metalloporphyrins, zinc, clofibrate and prebiotics in hyperbilirubinaemia, and EPO, folate, iron and vitamin E in anaemia. These alternative agents have varyingly been studied, but lack evidence to support their use. Although long-term outcome in HDFN seems favourable, neonates with foetal hydrops have a general bad outcome. This emphasises the need for adequate screening and treatment, preferably regulated by nationwide guidelines and monitoring.

Expert Commentary

Our clinical experience, as a tertiary referral centre of Neonatology, is in line with the conclusions reported above. The Leiden University Medical Centre is the national referral centre for HDFN in the Netherlands, which allowed us to develop as an expertise centre and to play an important role nationwide in the care for these neonates. Despite major advances in the field of HDFN, multiple issues still need to be addressed in the future to optimise the postnatal management of neonates with HDFN.

Firstly, it is important to emphasise that major improvements could probably be achieved by strict adherence to guidelines. Clear guidelines on screening and management appear to have significant effect, but have to be evaluated continuously and implemented around the globe. Strict adherence to guidelines is recommended to further eradicate kernicterus and treat affected foetuses and neonates effectively.

Secondly, to further assess the extent of the problems in the management of HDFN, it is important to assess its epidemiologic aspect. We need exact and national numbers on the occurrence of hyperbilirubinaemia, exchange transfusions and kernicterus as well as insight in clinical course and pitfalls in these cases. To better asses these cases, the pathophysiologic mechanisms also have to be better understood. For example, not much is known on BIND and why some infants develop kernicterus and others with an equally high serum bilirubin do not. It is also unclear what the exact mechanism of late anaemia is and why some neonates require top-up transfusions up to three months of life.

Thirdly, pharmacotherapeutic agents should be further researched as potential alternatives for and supplements to classical treatments. As pointed out, the role of albumin supplementation (prior to exchange transfusion), phenobarbital (especially antenatal maternal administration), metalloporphyrins, zinc, clofibrate and prebiotics in hyperbilirubinaemia needs to be clarified. Prospective, double-blinded RCTs are necessary to evaluate outcome and adverse effects, ideally in HDFN specific patient groups. The use of immunoglobulins in Rh haemolytic disease should be re-evaluated on efficacy and safety in the prevention of exchange transfusions. Larger trials are warranted to evaluate the use of EPO, folate and vitamin E in neonates with HDFN in the treatment of postnatal anaemia. Due to the low occurrence rate of HDFN, these trials should be multicentre international studies to reach sufficient power.

Lastly, there is a general lack of long-term follow-up studies in HDFN. Especially complications such as thrombocytopenia, iron overload and cholestasis should be studied in terms of short-term and long-term effects.

The ultimate goal in HDFN is timely detection and treatment of maternal alloimmunisation in an effort to lower the occurrence of hyperbilirubinaemia and to completely eradicate kernicterus, with a prosperous long-term prognosis for all affected neonates.

Five-year view

Detection and management of HDFN has vastly improved in the last decades. Unfortunately, it is still a major cause of hyperbilirubinaemia and, on occasion, kernicterus. The eradication of kernicterus has been a worldwide point of interest and can partly be achieved by adequate management of neonates with HDFN. By strict compliance to national guidelines, HDFN can be timely recognised and treated. It would be desirable in the future to obtain more information of the associations between serum bilirubin levels and neurologic damage, as well as epidemiologic information. Much attention is currently focused at identifying new pharmacological agents in HDFN. Some of these have already been used in clinical practice; others are currently under evaluation or await proper designed trials. Studies are needed on the impact of treatment in the long-term follow-up.

Key issues

- Despite RhD immunoprophylaxis, 1 to 3 in 1000 RhD negative woman develop anti-D antibodies, therefore HDFN is still a relevant condition of which treatment needs to be optimised.
- Guidelines for hyperbilirubinaemia and specifically for this group should be implemented worldwide and strictly followed.
- Intensive phototherapy and exchange transfusions are the treatment of choice in haemolytic hyperbilirubinaemia, although alternative pharmacotherapeutic agents are researched.
- Late anaemia is a common problem in neonates with HDFN and can last up to three months of age. In Rh haemolytic disease the majority of neonates needs at least one to up to 6 top-up transfusions.
- HDFN is associated with postnatal thrombocytopenia, iron overload and cholestasis.
- Limited studies are available on long-term outcome in HDFN, but generally show a favourable outcome in neonates that did not develop kernicterus.

References

1. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Hemolytic disease of the fetus and newborn. Vox Sang 2015;109:99-113.
2. Urbaniak SJ, Greiss MA. RhD hemolytic disease of the fetus and the newborn. Blood Rev 2000;14:44-61.
3. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med 2008;13:207-214.
4. Bowman J. Thirty-five years of Rh prophylaxis. Transfusion 2003;43:1661-1666.
5. Koelewijn JM, de Haas M, Vrijkotte TG, Bonsel GJ, van der Schoot CE. One single dose of 200 microg of antenatal RhIG halves the risk of anti-D immunization and hemolytic disease of the fetus and newborn in the next pregnancy. Transfusion 2008;48:1721-1729.
6. Bennardello F, Coluzzi S, Curciarello G, Todros T, Villa S. Recommendations for the prevention and treatment of haemolytic disease of the foetus and newborn. Blood Transfus 2015;13:109-134.
7. Illanes S, Soothill P. Management of red cell alloimmunisation in pregnancy: the non-invasive monitoring of the disease. Prenat Diagn 2010;30:668-673.
8. Fan J, Lee BK, Wikman AT, Johansson S, Reilly M. Associations of Rhesus and non-Rhesus maternal red blood cell alloimmunization with stillbirth and preterm birth. Int J Epidemiol 2014;43(4):1123–1131.
9. Bhutani VK, Johnson-Hamerman L. The clinical syndrome of bilirubin-induced neurologic dysfunction. Semin Fetal Neonatal Med 2015;20:6-13.
10. American Academy of Pediatrics Subcommittee on H. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316.
11. Shapiro SM, Bhutani VK, Johnson L. Hyperbilirubinemia and kernicterus. Clin Perinatol 2006;33:387-410.
12. Maisels MJ, McDonagh AF. Phototherapy for neonatal jaundice. New Engl J Med 2008;358:920-928.
13. Stokowski LA. Fundamentals of phototherapy for neonatal jaundice. Adv Neonatal Care 2011;11:S10-21.
14. Vreman HJ, Wong RJ, Stevenson DK. Phototherapy: current methods and future directions. Semin Perinatol 2004;28:326-333.
15. Woodgate P, Jardine LA. Neonatal jaundice : phototherapy. BMJ Clin Evid 2015;0319.
16. Xiong T, Qu Y, Cambier S, Mu D. The side effects of phototherapy for neonatal jaundice: what do we know? What should we do? Eur J Pediatr 2011;170:1247-1255.
17. Newman TB, Wickremasinghe AC, Walsh EM, Grimes BA, McCulloch CE, Kuzniewicz MW. Retrospective Cohort Study of Phototherapy and Childhood Cancer in Northern California. Pediatrics 2016;137(6): e20151354.
18. Wickremasinghe AC, Kuzniewicz MW, Grimes BA, McCulloch CE, Newman TB. Neonatal Phototherapy and Infantile Cancer. Pediatrics 2016;137(6):e2015135.
19. Lai YC, Yew YW. Neonatal Blue Light Phototherapy and Melanocytic Nevus Count in Children: A Systematic Review and Meta-Analysis of Observational Studies. Pediatr Dermatol 2016;33:62-68.
20. Das RR, Naik SS. Neonatal hyperbilirubinemia and childhood allergic diseases: a systematic review. Pediatr Allergy Immunol 2015;26:2-11.
21. Maayan-Metzger A, Yosipovitch G, Hadad E, Sirota L. Transepidermal water loss and skin hydration in preterm infants during phototherapy. Am J Perinatol 2001;18:393-396.

22. Boo NY, Lee HT. Randomized controlled trial of oral versus intravenous fluid supplementation on serum bilirubin level during phototherapy of term infants with severe hyperbilirubinaemia. J Paediatr Child Health 2002;38:151-155.

23. Mehta S, Kumar P, Narang A. A randomized controlled trial of fluid supplementation in term neonates with severe hyperbilirubinemia. J Pediatr 2005;147:781-785.

24. Diamond LK, Allen FH, Jr., Thomas WO, Jr. Erythroblastosis fetalis. VII. Treatment with exchange transfusion. N Engl J Med 1951;244:39-49.

25. Murki S, Kumar P. Blood exchange transfusion for infants with severe neonatal hyperbilirubinemia. Semin Perinatol 2011;35:175-184.

26. Dennery PA, Seidman DS, Stevenson DK. Neonatal hyperbilirubinemia. N Engl J Med 2001;344:581-590.

27. Chitty HE, Ziegler N, Savoia H, Doyle LW, Fox LM. Neonatal exchange transfusions in the 21st century: a single hospital study. J Paediatr Child Health 2013;49:825-832.

28. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal exchange transfusions and its effect on exchange-related morbidity and mortality. Pediatrics 2007;120:27-32.

29. Patra K, Storfer-Isser A, Siner B, Moore J, Hack M. Adverse events associated with neonatal exchange transfusion in the 1990s. J Pediatr 2004;144:626-631.

30. Abbas W, Attia NI, Hassanein SM. Two-stage single-volume exchange transfusion in severe hemolytic disease of the newborn. J Matern Fetal Neonatal Med 2012;25:1080-1083.

31. Thayyil S, Milligan DW. Single versus double volume exchange transfusion in jaundiced newborn infants. Cochrane Database Syst Rev 2006:CD004592.

32. Smits-Wintjens VEHJ, Walther FJ, Rath ME, Lindenburg ITM, te Pas AB, Kramer CM, Oepkes D, et al. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011;127:680-686.

33. De Boer IP, Zeestraten EC, Lopriore E, van Kamp IL, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008;198:54e1-4.

34. Gottstein R, Cooke RW. Systematic review of intravenous immunoglobulin in haemolytic disease of the newborn. Arch Dis Child Fetal Neonatal Ed 2003;88:F6-10.

35. Ballow M. The IgG molecule as a biological immune response modifier: mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory disorders. J Allergy Clin Immunol 2011;127:315-323;quiz 24-5.

36. Santos MC, Sa C, Gomes SC, Jr., Camacho LA, Moreira ME. The efficacy of the use of intravenous human immunoglobulin in Brazilian newborns with rhesus hemolytic disease: a randomized double-blind trial. Transfusion 2013;53:777-782.

37. Walsh S, Molloy EJ. Towards evidence based medicine for paediatricians. Is intravenous immunoglobulin superior to exchange transfusion in the management of hyperbilirubinaemia in term neonates? Arch Dis Child 2009;94:739-741.

38. Figueras-Aloy J, Rodriguez-Miguelez JM, Iriondo-Sanz M, Salvia-Roiges MD, Botet-Mussons F, Carbonell-Estrany X. Intravenous immunoglobulin and necrotizing enterocolitis in newborns with hemolytic disease. Pediatrics 2010;125:139-144.

39. Yang Y, Pan JJ, Zhou XG, Zhou XY, Cheng R, Hu YH. The effect of immunoglobulin treatment for hemolysis on the incidence of necrotizing enterocolitis - a meta-analysis. Eur Rev Med Pharmacol Sci 2016;20:3902-3910.

40. Louis D, More K, Oberoi S, Shah PS. Intravenous immunoglobulin in isoimmune haemolytic disease of newborn: an updated systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 2014;99:F325-331.
41. Shahian M, Moslehi MA. Effect of albumin administration prior to exchange transfusion in term neonates with hyperbilirubinemia--a randomized controlled trial. Indian Pediatr 2010;47:241-244.
42. Dash N, Kumar P, Sundaram V, Attri SV. Pre exchange Albumin Administration in Neonates with Hyperbilirubinemia: A Randomized Controlled Trial. Indian Pediatr 2015;52:763-767.
43. Kaplan M, Wong RJ, Sibley E. Neonatal-Perinatal Medicine. 9th ed. Cleveland (OH): Mosby; 2011.
44. Yaffe SJ, Levy G, Matsuzawa T, Baliah T. Enhancement of glucuronide-conjugating capacity in a hyperbilirubinemic infant due to apparent enzyme induction by phenobarbital. N Engl J Med 1966;275:1461-1466.
45. Kaabneh MA, Salama GS, Shakkoury AG, Al-Abdallah IM, Alshamari A, Halaseh RA. Phenobarbital and Phototherapy Combination Enhances Decline of Total Serum Bilirubin and May Decrease the Need for Blood Exchange Transfusion in Newborns with Isoimmune Hemolytic Disease. Clin Med Insights Pediatr 2015;9:67-72.
46. Trevett TN, Jr., Dorman K, Lamvu G, Moise KJ, Jr. Antenatal maternal administration of phenobarbital for the prevention of exchange transfusion in neonates with hemolytic disease of the fetus and newborn. Am J Obstet Gynecol 2005;192:478-482.
47. Stevenson DK, Rodgers PA, Vreman HJ. The use of metalloporphyrins for the chemoprevention of neonatal jaundice. Am J Dis Child 1989;143:353-356.
48. Suresh GK, Martin CL, Soll RF. Metalloporphyrins for treatment of unconjugated hyperbilirubinemia in neonates. Cochrane Database Syst Rev 2003:CD004207.
49. Méndez-Sánchez N, Roldán-Valadez E, Flores MA, Cardenas-Vazquez R, Uribe M. Zinc salts precipitate unconjugated bilirubin in vitro and inhibit enterohepatic cycling of bilirubin in hamsters. Eur J Clin Invest 2001;31:773-780.
50. Mishra S, Cheema A, Agarwal R, Deorari A, Paul V. Oral zinc for the prevention of hyperbilirubinaemia in neonates. Cochrane Database Syst Rev 2015:CD008432.
51. Sharma D, Farahbakhsh N, Sharma P, Shastri S. Role of oral zinc supplementation for reduction of neonatal hyperbilirubinemia: a systematic review of current evidence. J Matern Fetal Neonatal Med 2016:1-10.
52. Fallah R, Islami Z, Lotfi SR. Single dose of 50 mg/kg clofibrate in jaundice of healthy term neonates: randomised clinical trial of efficacy and safety. Indian J Pediatr 2012;79:194-197.
53. Habibi M, Mahyar A, Ayazi P, Ahmadabadi F, Javadi A. The effect of clofibrate on hyperbilirubinemia of term neonates. Acta Med Iran 2012;50:21-25.
54. Gholitabar M, McGuire H, Rennie J, Manning D, Lai R. Clofibrate in combination with phototherapy for unconjugated neonatal hyperbilirubinaemia. Cochrane Database Syst Rev 2012;12:CD009017.
55. Armanian AM, Barekatain B, Hoseinzadeh M, Salehimehr N. Prebiotics for the management of hyperbilirubinemia in preterm neonates. J Matern Fetal Neonatal Med 2016;29:3009-3013.
56. Bisceglia M, Indrio F, Riezzo G, Poerio V, Corapi U, Raimondi F. The effect of prebiotics in the management of neonatal hyperbilirubinaemia. Acta Paediatr 2009;98:1579-1581.
57. Gamsu HR, Mullinger BM, Donnai P, Dash CH. Antenatal administration of betamethasone to prevent respiratory distress syndrome in preterm infants: report of a UK multicentre trial. Br J Obstet Gynaecol 1989;96:401-410.

58. Porto AM, Coutinho IC, Correia JB, Amorim MM. Effectiveness of antenatal corticosteroids in reducing respiratory disorders in late preterm infants: randomised clinical trial. BMJ 2011;342:d1696.

59. al-Alaiyan S, al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med 1999;27:112-115.

60. Strand C, Polesky HF. Delayed anemia in erythroblastosis fetalis. Minn Med 1972;55:439-441.

61. Smits-Wintjens VEHJ, Walther FJ, Lopriore E. Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. Semin Fetal Neonatal Med 2008;13:265-271.

62. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, van Kamp IL, Oepkes D, Walther FJ, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100:312-316.

63. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, Oepkes D, Walther FJ, Lopriore E. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010;99:65-70.

64. Millard DD, Gidding SS, Socol ML, MacGregor SN, Dooley SL, Ney JA, Stockman JA. Effects of intravascular, intrauterine transfusion on prenatal and postnatal hemolysis and erythropoiesis in severe fetal isoimmunization. J Pediatr 1990;117:447-454.

65. Farrar D, Airey R, Law GR, Tuffnell D, Cattle B, Duley L. Measuring placental transfusion for term births: weighing babies with cord intact. BJOG 2011;118:70-75.

66. Garabedian C, Rakza T, Drumez E, Poleszczuk M, Ghesquiere L, Wibaut B, Depoortere MH, et al. Benefits of Delayed Cord Clamping in Red Blood Cell Alloimmunization. Pediatrics 2016;137:e20153236.

67. McAdams RM. Delayed cord clamping in red blood cell alloimmunization: safe, effective, and free? Transl Pediatr 2016;5:100-103.

68. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004;24:105-131.

69. Mainie P. Is there a role for erythropoietin in neonatal medicine? Early Hum Dev 2008;84:525-532.

70. Zanjani ED, Ascensao JL, McGlave PB, Banisadre M, Ash RC. Studies on the liver to kidney switch of erythropoietin production. J Clin Invest 1981;67:1183-1188.

71. Aher SM, Ohlsson A. Early versus late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2006:CD004865.

72. Nicaise C, Gire C, Casha P, d'Ercole C, Chau C, Palix C. Erythropoietin as treatment for late hyporegenerative anemia in neonates with Rh hemolytic disease after in utero exchange transfusion. Fetal Diagn Ther 2002;17:22-24.

73. Pessler F, Hart D. Hyporegenerative anemia associated with Rh hemolytic disease: treatment failure of recombinant erythropoietin. J Pediatr Hematol Oncol 2002;24:689-693.

74. Zuppa AA, Alighieri G, Calabrese V, Visintini F, Cota F, Carducci C, Antichi E, et al. Recombinant human erythropoietin in the prevention of late anemia in intrauterine transfused neonates with Rh-isoimmunization. J Pediatr Hematol Oncol 2010;32:e95-101.

75. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2014:CD004863.

76. Haiden N, Klebermass K, Cardona F, Schwindt J, Berger A, Kohlhauser-Vollmuth C, Jilma B, et al. A randomized, controlled trial of the effects of adding vitamin B12 and folate to erythropoietin for the treatment of anemia of prematurity. Pediatrics 2006;118:180-188.

77. Carnielli VP, Da Riol R, Montini G. Iron supplementation enhances response to high doses of recombinant human erythropoietin in preterm infants. Arch Dis Child Fetal Neonatal Ed 1998;79:F44-48.

78. Berger HM, Lindeman JH, van Zoeren-Grobben D, Houdkamp E, Schrijver J, Kanhai HH. Iron overload, free radical damage, and rhesus haemolytic disease. Lancet 1990;335:933-936.

79. Rath ME, Smits-Wintjens VEHJ, Oepkes D, Walther FJ, Lopriore E. Iron status in infants with alloimmune haemolytic disease in the first three months of life. Vox Sang 2013;105:328-333.

80. Kling PJ, Schmidt RL, Roberts RA, Widness JA. Serum erythropoietin levels during infancy: associations with erythropoiesis. J Pediatr 1996;128:791-796.

81. Pathak A, Roth P, Piscitelli J, Johnson L. Effects of vitamin E supplementation during erythropoietin treatment of the anaemia of prematurity. Arch Dis Child Fetal Neonatal Ed 2003;88:F324-328.

82. Bousema S, Govaert P, Dudink J, Steegers EAP, Reiss IKM, de Jonge RCJ. Kernicterus is preventable but still occurs. Ned Tijdschr Geneeskd 2015;159(21):A8518. Dutch.

83. Hansen TWR. Kernicterus: an international perspective. Semin Neonatal 2002 ;7 :103-109.

84. Wusthoff CJ, Loe IM. Impact of bilirubin-induced neurologic dysfunction on neurodevelopmental outcomes. Semin Fetal Neonatal Med 2015;20:52-57.

85. Koenig JM, Christensen RD. Neutropenia and thrombocytopenia in infants with Rh hemolytic disease. J Pediatr 1989;114:625-631.

86. van den Akker ES, de Haan TR, Lopriore E, Brand A, Kanhai HH, Oepkes D. Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies. Am J Obstet Gynecol 2008;199:387e1-4.

87. Rath ME, Smits-Wintjens VEHJ, Oepkes D, van Zwet EW, van Kamp IL, Brand A, Walther FJ, et al. Thrombocytopenia at birth in neonates with red cell alloimmune haemolytic disease. Vox Sang 2012;102:228-233.

88. Aygun C, Tekinalp G, Gurgey A. Increased fetal iron load in rhesus hemolytic disease. Pediatr Hematol Oncol 2004;21:329-333.

89. Siddappa AM, Rao R, Long JD, Widness JA, Georgieff MK. The assessment of newborn iron stores at birth: a review of the literature and standards for ferritin concentrations. Neonatology 2007;92:73-82.

90. Allgood C, Bolisetty S. Severe conjugated hyperbilirubinaemia and neonatal haemolysis. Int J Clin Pract 2006;60:1513-1514.

91. Dunn PM. Obstructive Jaundice and Haemolytic Disease of the Newborn. Arch Dis Child 1963;38:54-61.

92. Smits-Wintjens VEHJ, Rath ME, Lindenburg IT, Oepkes D, van Zwet EW, Walther FJ, Lopriore E. Cholestasis in neonates with red cell alloimmune hemolytic disease: incidence, risk factors and outcome. Neonatology 2012;101:306-310.

93. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 1989;298:564-567.

94. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 2008;105:17046-17049.

95. Lindenburg ITM, van Klink JMM, Smits-Wintjens VEHJ, van Kamp IL, Oepkes D, Lopriore E. Long-term neurodevelopmental and cardiovascular outcome after intrauterine transfusions for fetal anaemia: a review. Prenat Diagn 2013;33:815-822.

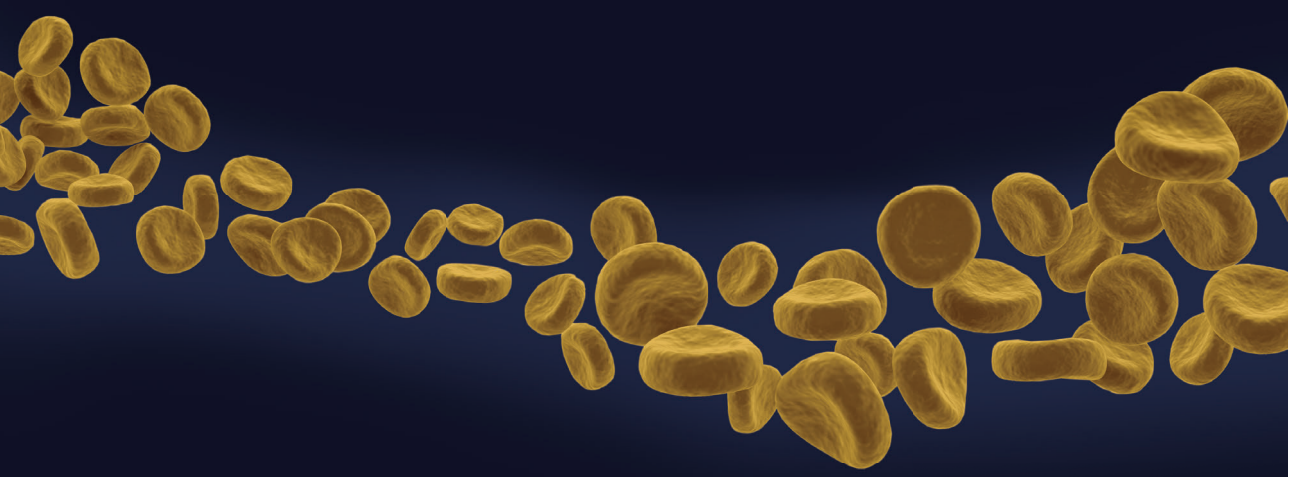
96. Lindenburg ITM, Smits-Wintjens VEHJ, van Klink JMM, Verduin E, van Kamp IL, Walther FJ, Schonewille H, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am J Obstet Gynecol 2012;206:141e1-8.

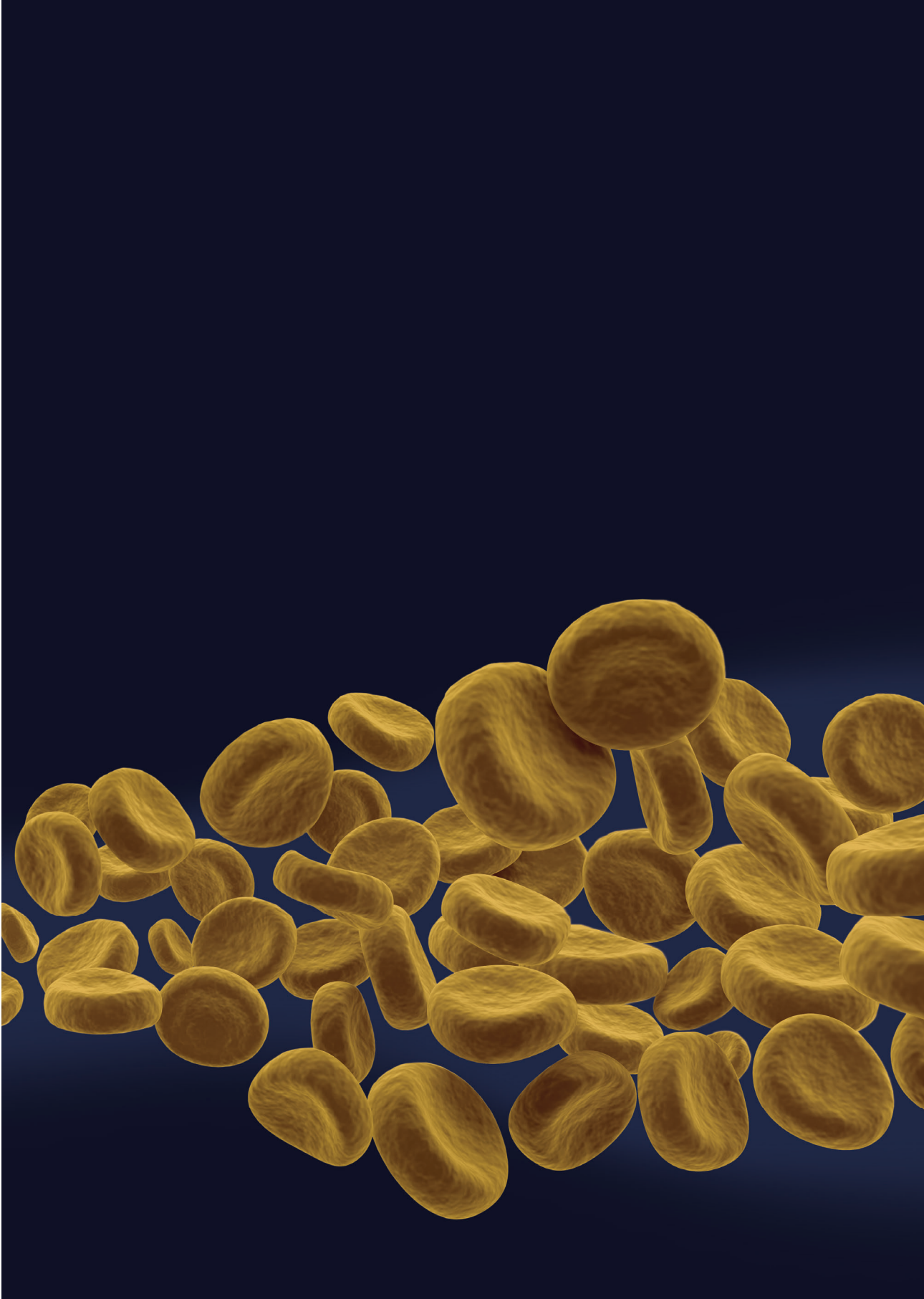
97. van Klink JMM, Lindenburg ITM, Inklaar MJ, Verduin E, Koopman HM, van Kamp IL, Schonewille H, et al. Health-Related Quality of Life and Behavioral Functioning after Intrauterine Transfusion for Alloimmune Anemia. J Pediatr 2015;167:1130-1135e2.

98. Wallace AH, Dalziel SR, Cowan BR, Young AA, Thornburg KL, Harding JE. Long-term cardiovascular outcome following fetal anaemia and intrauterine transfusion: a cohort study. Arch Dis Child 2017;102(1):40-45.

99. van Klink JMM, van Veen SJ, Smits-Wintjens VEHJ, Lindenburg ITM, Rijken M, Oepkes D, Lopriore E. Immunoglobulins in Neonates with Rhesus Hemolytic Disease of the Fetus and Newborn: Long-Term Outcome in a Randomized Trial. Fetal Diagn Ther 2016;39:209-213.

Part 2 - Predictors of severe disease





Chapter two

Are foetal bilirubin levels associated with the need for neonatal exchange transfusions in haemolytic disease of the foetus and newborn?

Isabelle M.C. Ree
Renske M. van ’t Oever
Carolien Zwiers
Joanne (E.)J.T. Verweij
Dick Oepkes
Masja de Haas
Enrico Lopriore

American Journal of Obstetrics and Gynecology MFM 2021;18;3(3):100332.

Abstract

Background

Foetal bilirubin is routinely measured at our centre when taking a pretransfusion blood sample at intrauterine transfusions in haemolytic disease of the foetus and newborn. However, the clinical value of foetal bilirubin assessment is not well known, and the information is rarely used. We speculated that there could be a role for this measurement in predicting the need for neonatal exchange transfusion.

Objective

To evaluate the predictive value of foetal bilirubin for exchange transfusions in severe haemolytic disease of the foetus and newborn.

Study design

A total of 186 infants with Rh alloantibody-mediated haemolytic disease of the foetus and newborn, treated with one or more intrauterine transfusions at the Leiden University Medical Centre between 2006 and June 2020, were included in this observational study. Antenatal and postnatal factors were compared between infants with and without exchange transfusion treatments. The primary outcome was the foetal bilirubin level before the last intrauterine transfusion in relation to the need for exchange transfusion.

Results

In a multivariate logistic regression analysis, the foetal bilirubin level before the last intrauterine transfusions (odds ratio 1.32; 95%-confidence interval 1.09-1.61 per 1 mg/dL) and the total number of IUTs (odds ratio 0.63; 95%-confidence interval 0.44-0.91 per intrauterine transfusion) were independently associated with the need for exchange transfusion. The area under the curve was determined at 0.71. A Youden index was calculated of 0.43. The corresponding foetal bilirubin level was 5 mg/dL and had a sensitivity of 79% and a specificity of 64%.

Conclusion

A high foetal bilirubin level before the last intrauterine transfusion was associated with a high likelihood of neonatal exchange transfusion.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which maternal alloantibodies lead to the destruction of foetal erythrocytes. In the antenatal period, this can lead to severe foetal anaemia, foetal hydrops and ultimately intrauterine demise.[1,2] The mainstay of antenatal treatment in HDFN is intrauterine transfusion (IUT) to correct foetal anaemia. In the postnatal period, HDFN can lead to severe hyperbilirubinaemia and prolonged anaemia. Hyperbilirubinaemia may cause kernicterus or chronic bilirubin encephalopathy, which can lead to permanent brain damage.[1] Hyperbilirubinaemia is primarily treated by intensive phototherapy and, in case of treatment failure, exchange transfusion (ET) to rapidly excrete excess bilirubin.

ET is an invasive procedure with rates of associated morbidity up to 74%. Complications include central line complications (such as catheter infection, thrombosis or dislocation), thrombocytopenia and neutropenia, as well as metabolic derangement.[3.] The rate of ET in infants with severe HDFN treated at our centre was approximately 15% after more restrictive guideline adaptation from the American Academy of Paediatrics (AAP) in 2005.[4] Although a few potential variables have been identified as risk factors for the need for ET, it is still challenging to predict antenatally which infant will likely require ET. Improved antenatal prediction could help anticipate and improve individualised postnatal care.

As foetal bilirubin levels are routinely measured at our centre before each IUT procedure and high bilirubin levels after birth are indicators for ET, we hypothesised that high levels of foetal bilirubin before birth could predict the need of ET in the neonatal period. The primary aim of this study was to evaluate whether foetal bilirubin could be a predictor for ET in the neonatal period.

Materials and Methods

Study design and population

All children treated with one or more IUT(s) for severe Rh-mediated HDFN between January 2006 and June 2020 at the Leiden University Medical Centre (LUMC) were included in an observational study. The LUMC is the national referral centre for maternal-foetal therapy in the Netherlands for treatment of severe foetal anaemia caused by red blood cell alloimmunisation. After foetal treatment with IUT, admission of the neonate to the LUMC is highly recommended;

therefore, the study was composed of nearly all live-born infants treated with IUT for HDFN in the Netherlands during the study period. Incidentally, infants were admitted elsewhere do to spontaneous preterm delivery. Furthermore, we included only infants treated with IUT as foetal samples of bilirubin are only taken during this procedure and not in cases without IUT. The cohort was selected from 2006 onwards as ET and phototherapy thresholds were changed in 2005. After the guideline change, the criteria for ET were as follows: (1) total serum bilirubin above thresholds of 0.5 mL/dL/h according to the AAP guideline; (2) rise of bilirubin >0.5 ml/dL/h despite intensive phototherapy; and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level.[4] Infants born at <35 weeks' gestation were excluded in this study, as well as infants with blood group alloimmunisation caused by non-Rh antigens because of different pathophysiological characteristics in terms of bilirubin accumulation and ET risk after birth.[5]

Data collection

Patient data were collected from medical records of all infants and anonymously recorded. Collected data included: sex, gestational age (GA) at birth (weeks), birth weight, caesarean delivery, GA at first IUT (weeks), number of IUTs per foetus, type of alloimmunisation, number of ETs per infant, hours after birth until first ET, bilirubin level at birth, maximum bilirubin level after birth, and foetal bilirubin level at all IUTs. Because of the non-invasive nature of this study, the medical ethics committee of our centre granted a waiver of consent.

Outcome measures

The primary outcome was the bilirubin level before the last IUT of infants treated with ET in the neonatal period, compared with the bilirubin level before the last IUT of infants not treated with ET. Secondary outcomes included the number of ETs per infant, time from birth to first ET (hours), and number of days of phototherapy.

Statistical analysis

The following variables were compared between infants with and without treatments with ET as potential predictors of ET: sex, GA at birth, number of days between birth and last IUT, total number of IUTs, bilirubin at birth, and foetal bilirubin at last IUT. In addition, these variables were included in a multivariable logistic regression model, to correct for potential confounders, except for the bilirubin at birth as this value is highly correlated with the foetal bilirubin at last IUT. Results are presented as odds ratios (ORs) with 95%-confidence intervals (95% CIs). A receiver operating characteristic (ROC) curve was made, and the result is presented as the

area under the curve (AUC). To find the cut-off value for foetal bilirubin as a predictor for ET, the Youden index was calculated. The Youden Index is presented as an absolute foetal bilirubin level with corresponding sensitivity and specificity. Statistical analyses were performed using IBM SPSS Statistics (version 26.0; SPSS Inc, Chicago, IL).

Results

During the study period, 403 infants were born with HDFN and admitted to the LUMC, of which 207 were treated with IUT during the study period and thus were eligible for this study. A total of 84 infants were excluded, as HDFN was caused by a non-Rh antigen in 60 infants and an additional 24 infants were excluded due to a GA of <35 weeks. The derivation of the study population is shown in Figure 1. The baseline characteristics of the study group are presented in Table 1.

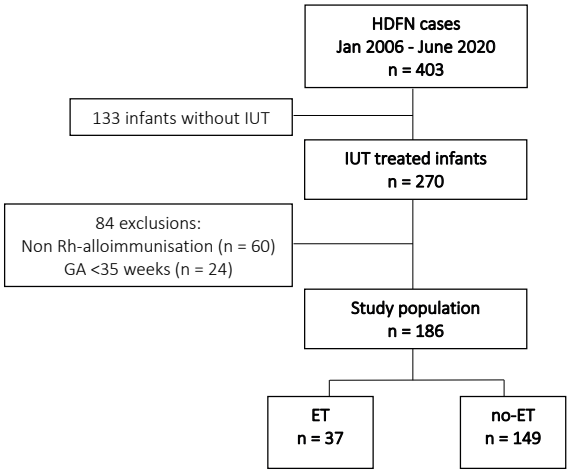


Figure 1. Flowchart of the study population
ET, exchange transfusion; *GA*, gestational age; *HDFN*, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion.

Table 1. Baseline characteristics

	Infants treated with IUT(s), n = 186
Male - n (%)	107 (58)
Gestational age at birth (weeks) - median (IQR)	36 (36-37)
Birth weight (g) - mean ± SD	2869 ± 362
Gestational age at first IUT (weeks) - median (IQR)	29 (25-32)
Number of IUTs per foetus - median (IQR)	2.0 (1.0-3.3)
Type of alloimmunisation[a]	
D alloimmunisation - n (%)	174 (94)
C alloimmunisation - n (%)	1 (1)
c alloimmunisation - n (%)	10 (5)
E alloimmunisation - n (%)	1 (1)

IQR, interquartile range*; IUT*, intrauterine transfusion;w *n*, number; *SD*, standard deviation.
Data are presented as mean ± *SD,* median (*IQR*), or n with percentage (%).
[a] Percentage value does not add up to 100% because of rounding.

Here, 37 infants (20%) received an ET after birth. In the ET group, the median number of ET per infant was 1 with a median of 24 hours after birth until the first ET. The maximum bilirubin level after birth reached an average of 19 mg/dL.

Univariate analysis of potential predictors for ET was performed (Table 2). The level of foetal bilirubin at the last IUT was positively associated with an increased need for ET after birth (unadjusted OR 1.32; 95% CI 1.10-1.58 per 1 mg/dL). A higher number of IUTs received was associated with a reduced need for ET (OR 0.65; 95% CI 0.47-0.90 per IUT).

T**able 2.** Predictors for exchange transfusions in infants with HDFN treated with intrauterine transfusions

	ET (n = 37)	**No ET (n = 149)**	**Univariable OR (95% CI)**	**Multivariable OR (95% CI)**
Foetal bilirubin at the last IUT (OR per 1 mg/dL)[a]	6.02 (5.18-7.09)	4.33 (3.27-6.20)	1.32 (1.10-1.58)	1.32 (1.09-1.61)
Male - n (%)	26 (70)	81 (54)	0.50 (0.23-1.09)	1.52 (0.66-3.50)
Gestational age <37 weeks - n (%)	23 (62)	86 (58)	0.83 (0.40-1.74)	0.95 (0.41-2.19)
Number of days between birth and last IUT (OR per day)[a]	19 (15-24)	21 (19-26)	0.97 (0.93-1.01)	1.00 (0.96-1.03)
Number of IUTs (OR per IUT)[a]	2.0 (1.0-3.0)	2.0 (2.0-4.0)	0.65 (0.47-0.90)	0.63 (0.44-0.91)
Bilirubin at birth (OR per 1 mg/dL)[a]	7.89 (6.52-10.82)	5.47 (4.09-6.56)	1.72 (1.41-2.10)	-

CI, confidence interval; *HDFN*, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Data presented as median (*IQR*).

To assess the independent association of these risk factors on the need for ET after birth, the factors were entered in a multivariate logistic regression model (Table 2). Foetal bilirubin at last IUT (OR 1.32; 95% CI 1.09-1.61 per 1 mg/dL) and the number of IUTs (OR 0.63; 95% CI 0.44-0.91 per IUT) were still independently associated with the need for ET.

An ROC-curve was plotted and the AUC for foetal bilirubin was 0.71. The corresponding Youden Index was calculated at 0.43 with a cut-off foetal bilirubin level of 5 mg/dL with a sensitivity of 79% and a specificity of 64% (Figure 2). To visualise the course of foetal bilirubin, a boxplot of the foetal bilirubin level at each IUT was computed (Figure 3). The figure shows a small and statistically insignificant decline in foetal bilirubin over the course of IUTs.

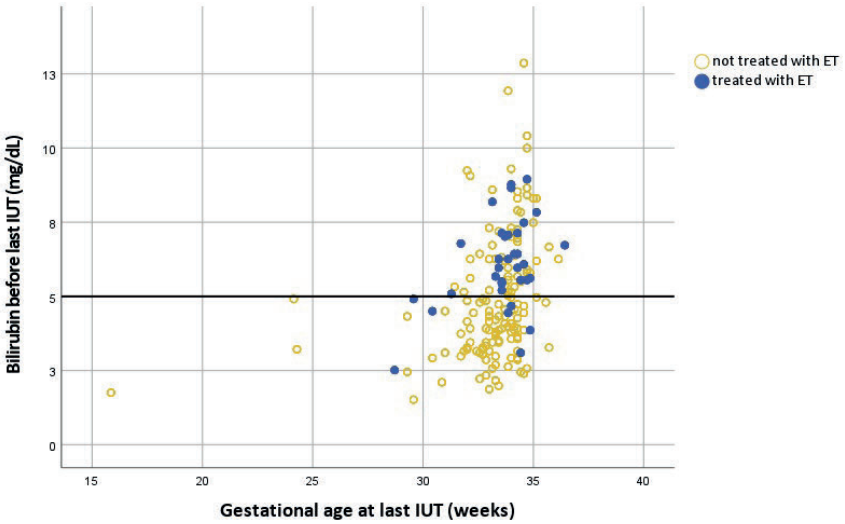


Figure 2. Absolute bilirubin levels per foetus before last IUT
ET, exchange transfusion; *IUT*, intrauterine transfusion.
The *line* is plotted at the bilirubin value of 5 mg/dL, the value with the highest sensitivity and specificity in predicting ET (the Youden index).

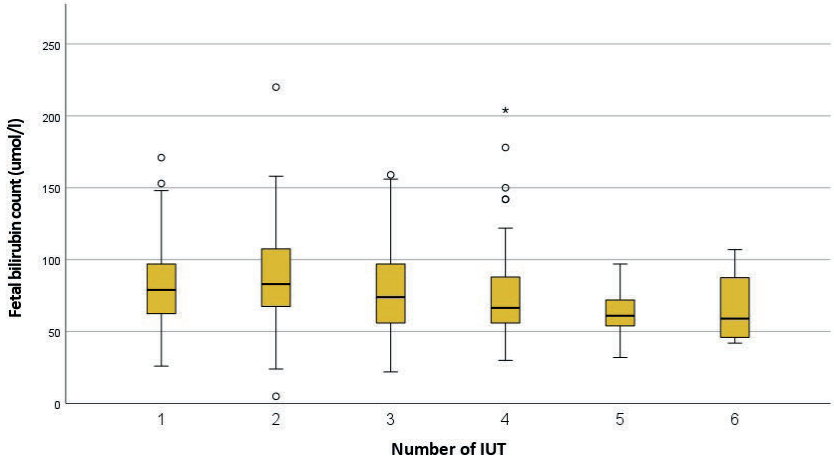


Figure 3. Boxplot of foetal bilirubin levels throughout IUT treatment
IQR, interquartile range; *IUT*, intrauterine transfusion.
Boxplots display median values with IQR, *dots* represent outliers, and *asterisks* represent extreme outliers defined as 3 times the IQR.

Discussion

Principal Findings

This study shows that foetal bilirubin measured at the last IUT treatment has a predictive value for the need for ET after birth. 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 ET treatment after birth. An additional finding in this study was that a higher number of IUTs decreases the need for ET. This finding confirmed previous reports from our study group.[6] The cumulative protective effect of IUTs on ET treatment was hypothesised to be caused by the replacement of foetal, incompatible blood by compatible donor blood and thus interference with the haemolytic process, causing less bilirubin to accumulate and a longer blood transfusion-free interval after birth.[6]

Here, the rate of ET among infants treated with IUT is 20%; the rate was in line with a general international ET decline over the past 20 years. Among all infants with HDFN, the rate of ET at our centre declined from 67% (200-2005) to 10% (2015-2000).[7]

Clinical Implications

High foetal bilirubin levels could be viewed as an early, foetal predictor for the need for ET. Combined with other risk factors, it could potentially be used to determine a personal risk profile for infants affected by severe HDFN to predict more accurately the neonatal course and plan neonatal care and follow-up accordingly.

We speculated that higher bilirubin levels in the ET group could indicate a stronger haemolytic process with continued production of foetal cells, explaining the correlation with ET treatment after birth. Other possible explanations could be related to impaired placenta function or perhaps saturation of maternal bilirubin excretion capacity.

Research Implications

Above all, the findings of this study add to the pathophysiological understanding of HDFN and foetal bilirubin metabolism in relation to IUT treatment.

The mechanism of bilirubin metabolism in infants is well known,[8] but foetal bilirubin metabolism is less well understood. The foetal liver is poorly capable of conjugating bilirubin and is suggested to have an excretory defect so that even if the foetus could conjugate bilirubin, it could not excrete it.[9] Animal studies in guinea pigs and monkeys show that unconjugated bilirubin can diffuse freely over the placenta and, due to the gradient in favour of the transport from foetal side to maternal side, foetal unconjugated bilirubin is cleared from foetal circulation and transported to the maternal circulation where it is conjugated and excreted.[9,10] In addition, in vitro experiments provided evidence for the existence of carrier-mediated systems for transport of unconjugated bilirubin across the plasma membranes of human placental trophoblast in addition to transport by diffusion.[11,12] None of these studies mention the (possibly harmful) effect of high foetal bilirubin levels on the foetus or provide foetal bilirubin reference values. If basal ganglia are just as susceptible to bilirubin toxicity during foetal life as in the neonatal period, high levels of foetal bilirubin could lead to increased risk of brain injury and impaired neurodevelopmental outcome. More research is needed to address this hypothesis.

Strengths and Limitations

One of the strengths of our study was that because our centre is the national referral centre for HDFN, we have a uniformly treated cohort, limiting variability in treatment of HDFN. A limitation to this study is the relatively small sample size; however, it is the largest homogenous

cohort to date to address this subject. Finally, we could not include infants with HDFN without IUTs in this study, despite 15% of these infants was treated with ET at our centre in the same period. As these infants did not receive IUT treatment, no foetal blood sampling was available in this group.

Conclusions

Our study demonstrated the possibility of using foetal bilirubin levels as a predictor for ET, making it possible to further accommodate adequate and individualised neonatal care. To translate this study to the clinical setting, more research is necessary, including prospective validation. Studies should focus on computing a model that incorporates multiple risk factors such as foetal bilirubin, to create personalised risk and treatment profiles for infants affected by severe HDFN.

References

1. Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. Expert Rev Hematol 2017;10(7):607-616.
2. Delaney M, Matthews DC, Hemolytic disease of the fetus and newborn: managing the mother, fetus, and newborn. Hematology Am Soc Hematol Educ Program 2015;146-151.
3. Smits-Wintjens VE, Rath ME, van Zwet EW, Oepkes D, Brand A, Walther FJ, Lopriore E. Neonatal morbidity after exchange transfusion for red cell alloimmune hemolytic disease. Neonatology 2013;103(2):141-147.
4. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316.
5. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, van Kamp IL, Oepkes D, Walther FJ, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100(3):312-316.
6. Ree IMC, Lopriore E, Zwiers C, Böhringer S, Janssen MWM, Oepkes D, De Haas M. Suppression of compensatory erythropoiesis in hemolytic disease of the fetus and newborn due to intrauterine transfusions. Am J Obstet Gynecol 2020;223(1):119.e1-10.
7. Ree IMC, Besuden CFJ, Wintjens VEHJ, Verweij EJT, Oepkes D, De Haas M, Lopriore E. Exchange transfusions in severe Rh-mediated alloimmune haemolytic disease of the foetus and newborn - A 20-year overview on the incidence, associated risks and outcome. Vox Sang 2021;116(9):990-997.
8. Dennery PA, Seidman DS, Stevenson DK. Neonatal hyperbilirubinemia. N Engl J Med 2001;22;344(8):581-590.
9. Schenker S, Dawber NH, Schmid R. Bilirubin Metabolism in the Fetus. J Clin Invest 1964;43:32-39.
10. Lester R, Behrman RE, Lucey IF. Transfer of Bilirubin-C14 Across Monkey Placenta. Pediatrics 1963;32:416-419.
11. Serrano MA, Bayón JE, Pascolo L, Tiribelli C, Ostrow JD, Gonzalez-Gallego J, Marin JJG. Evidence for carrier-mediated transport of unconjugated bilirubin across plasma membrane vesicles from human placental trophoblast. Placenta 2002;23(7):527-535.
12. Rigato I, Pascolo L, Fernetti C, Ostrow JD, Tiribelli C. The human multidrug-resistance-associated protein MRP1 mediates ATP-dependent transport of unconjugated bilirubin. Biochem J 2004;383(Pt 2):335-341.

Chapter three

Suppression of compensatory erythropoiesis in haemolytic disease of the foetus and newborn due to intrauterine transfusions

Isabelle M.C. Ree
Enrico Lopriore
Carolien Zwiers
Stefan Böhringer
Marleen W.M. Janssen
Dick Oepkes
Masja de Haas

American Journal of Obstetrics and Gynecology 2020;223(1):119.e1-10.

Abstract

Background

Infants with severe haemolytic disease of the foetus and newborn (HDFN) often require one or multiple intrauterine transfusions (IUTs) to treat foetal anaemia. IUTs may have an inhibiting effect on foetal and neonatal erythropoiesis.

Objective

To quantify the effect of one or multiple IUTs on the foetal erythropoiesis by assessing the foetal reticulocyte counts in a population with severe HDFN.

Study design

This was an observational cohort study in infants admitted to the Leiden University Medical Centre (LUMC) who received one or multiple IUT(s) for HDFN caused by (Rh)D or K antibodies that were born between January 2005 and December 2018.

Results

A total of 235 patients were included, of whom 189 patients with D-mediated HDFN and 46 with K-mediated HDFN. Absolute foetal reticulocyte count in D-mediated HDFN declined exponentially over the course of consecutive IUTs, with a 62% decline after one IUT (95%-confidence interval 56-67). A similar exponential decline was observed in K-mediated HDFN, with 32% (95%-confidence interval 19-45) decline after one IUT. This decline was not associated with the varying gestational age at the time of the first IUT or the total number of IUTs. The number of red blood cell transfusions for postnatal anaemia was greater for infants with D- and K-mediated HDFN with >2 IUTs (median of 3 [interquartile range 2-3] vs 2 [interquartile range 1-3], p=.035 in D-mediated disease and median of 2 [interquartile range 1-2] vs 1 [interquartile range 1-1], p<.001 in K-mediated disease). Infants born after >2 IUTs less often required exchange transfusion in D-mediated HDFN (19/89 [21%] vs 31/100 [31%], p=.039), compared to infants with 1-2 IUTs.

Conclusion

Treatment with IUTs causes an exponential decrease in foetal reticulocyte counts in both D- and K-mediated HDFN. Suppression of the compensatory erythropoiesis leads to prolonged postnatal anaemia and an increased requirement of red blood cell transfusions after birth.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is caused by an incompatibility between maternal and foetal erythrocyte antigens. HDFN is characterised by foetal and neonatal erythroid cell destruction due to maternal alloantibodies, which will induce compensatory erythropoiesis. In case of insufficient compensation, foetal or neonatal anaemia may occur and intrauterine treatment with one or more red blood cell (RBC) transfusions may be indicated, as well as transfusions for persistent anaemia after birth.[1,2] The exact effects of intrauterine transfusions (IUTs) with adult donor RBCs, which carry a different type of haemoglobin and have different oxygen binding and release characteristics compared to foetal red cells, is not known. In small populations, the effect of IUT(s) on various haematologic parameters such as foetal haematocrit, haemoglobin, leukocytes and bilirubin has been studied.[3-5] Reticulocyte counts at birth appeared to be lower in infants with HDFN who received one or multiple IUTs, compared with infants that were not treated with IUTs, irrespective of the haemoglobin level at birth.[3,6] Treatment with IUTs is also associated with a higher number of transfusions after birth compared to infants with HDFN not treated with IUT(s).[7] An inhibiting effect of donor blood on the foetal and neonatal erythropoiesis has been postulated before.[3] The total number of IUTs per infant may be of clinical relevance to select infants at increased risk for a complicated postnatal course.

A wider understanding of the effects of IUTs on foetal and neonatal erythropoiesis is necessary in order to clarify the pathophysiological mechanisms underlying both the intrauterine and postnatal course of HDFN. In this study, we specifically aimed to quantify the effect of one or multiple IUTs on foetal erythropoiesis by assessing the reticulocyte counts in a large population of foetuses and infants with severe HDFN.

Methods

Study population

All infants admitted to the Leiden University Medical Centre (LUMC) who received treatment with one or multiple IUT(s) for the treatment of HDFN caused by (Rh)D or K antibodies and who were born between January 2005 and December 2018, were eligible for the study. In the Netherlands, all pregnant women are routinely screened for the presence of alloantibodies in pregnancy and maternal blood samples with a positive screening result are sent to one of the two national referral laboratories (Sanquin Diagnostic Services or the Special Institute for

Blood group Investigations). Thereafter, the clinical relevance of the antibody is evaluated by assessing the antibody specificity, and by assessing whether the foetus is antigen-positive. If the foetus is positive, the risk on foetal haemolysis is assessed by serially determining the antibody titre and antibody-dependent cell-mediated cytotoxicity (ADCC). Referral to the LUMC as national specialised centre is indicated if laboratory parameters are above determined cut-offs. These cut-offs are antibody titres tested in maternal serum ≥16 in D alloimmunisation and ≥2 in K alloimmunisation, or in case of an ADCC assay ≥50% in D alloimmunisation and ≥30% In Kell alloimmunisation. Subsequently, these high-risk pregnancies are monitored by serial Doppler measurements to assess the velocity of the blood flow in the middle cerebral artery (MCA). If MCA Doppler exceeds 1.5 multiples of the median or if signs of hydrops are present, treatment with a first or subsequent IUT is indicated. One or more IUTs can be administered until 34-35 weeks' gestation, after which induced delivery is preferred to IUT treatment. The IUT technique used in the Netherlands has been previously described.[8] IUTs consist of irradiated, Parvovirus B19 and Cytomegalovirus seronegative packed erythrocytes, with an increased haematocrit of 0.80-0.85 L/L to minimise the risk of volume overload in the foetus. IUTs are preferably administered intravascularly, either into the placental cord insertion or into the intrahepatic part of the umbilical vein (often in combination with additional intraperitoneal transfusion), depending on the orientation of the placenta. To confirm the suspected foetal anaemia, a foetal blood sample is taken prior to the procedure. Planned delivery at the LUMC and neonatal admission to the neonatal intensive care unit of the LUMC is recommended for all pregnancies after IUT.

Infant and foetal data were excluded from analyses in case of HDFN caused by other alloantibodies than D or K, and major congenital malformations. Unsuccessful IUTs were defined as transfusion with a volume of less than 5 mL, as the pre-IUT blood sample is 5 mL, and were excluded.

In HDFN mediated by K antibodies, IUTs are generally needed earlier in gestation compared to D-mediated disease and erythroid suppression seems to be the predominant mechanism in producing foetal anaemia rather than haemolysis.[9-11] The results of infants with D- and K-mediated HDFN were therefore reported separately.

Data collection

Data was extracted from the hospital's patient database, including maternal and neonatal medical files and laboratory outcomes. Follow-up data on transfusions after discharge from the LUMC were collected from referral hospitals. Written consent was obtained from

the parents or caregivers and all personal data was coded prior to analysis. The following maternal and foetal data were recorded: number of previous births, antenatal intravenous immunoglobulins administration, maximum antibody titre, maternal age at first IUT, foetal gestational age at each IUT, total number of IUTs, volume dosage of IUT, IUT procedure access site (placental cord insertion or intrahepatic transfusion with or without intraperitoneal transfusion), foetal haemoglobin levels and leukocyte, platelet, and reticulocyte counts before every IUT. The following infant data were recorded: sex, gestational age at birth, birth weight, haemoglobin level at birth, reticulocyte count at birth, bilirubin level at birth, number of days of phototherapy, treatment with exchange transfusion (ET), treatment with postnatal RBC transfusion(s) the first three months after birth, and the number of postnatal transfusion(s) per infant (also known as "top-up" transfusions).

Referral hospitals receive the protocol for postnatal transfusions of the LUMC after discharge to their centre to unify neonatal management. The current transfusion guideline of the department recommends a transfusion in term infants with HDFN when haemoglobin levels fall below 10.5 g/dL (6.5 mmol/L) for day 0-6, below 8.9 g/dL (5.5 mmol/L) for day 7-13, and below 7.2 g/dL (4.5 mmol/L) from day 14 onwards. A transfusion of 15 ml/kg irradiated packed erythrocytes less than 5 days old was advised throughout the study period, with a haematocrit of 0.50-0.65 L/L.

Exchange transfusion in the Netherlands is indicated within 24 hours after birth if the serum bilirubin level is above the cut-off values for exchange transfusion and proceeds to rise despite adequate intensive phototherapy (consisting of 4 phototherapy lamps), or if after 24 hours the bilirubin is above the cut-off values for exchange transfusion.

The study protocol and analysis plan were approved by the ethics committee of the LUMC (G19.041) and scientific committee of our department.

Primary and secondary outcome

The primary outcome in this study was the suppression of foetal erythropoiesis, as defined by the decline (%) in absolute reticulocyte count ($\cdot 10^9$/L) per consecutive IUT. The primary outcome was adjusted for the total number of IUTs per infant (as indicator of disease severity) and gestational age in weeks at time of the first IUT as measure for suppression of foetal erythropoiesis. The relative reticulocyte count, as expressed per thousand RBCs (‰), was also reported. Secondary outcomes were the change in leukocytes and platelets per IUT, the reticulocyte counts at birth (absolute and relative counts), ferritin levels at birth, the proportion

of neonates requiring ET, and the proportion of neonates requiring RBC transfusion(s) after birth.

Statistical analysis

Data is presented as mean (± standard deviation, SD) or median (interquartile range, IQR) depending on the underlying distribution. The primary outcome is visualised in a boxplot. A linear mixed model was performed to account for the fact of repeated measurements and allow for covariate adjustment. Effect sizes are reported together with 95%-confidence intervals (95% CI). The outcome variable of the model was the absolute reticulocyte count and the predicting variable the IUT number. The total number of IUTs per infant and gestational age in weeks at time of the first IUT were included as potential confounders. Data were log10-transformed to unskew the distribution. Changes on the linear scale for the log10-transformed values correspond to percentage changes on the raw scale. The change in leukocytes and platelets was assessed with a linear mixed model using a random intercept per individual. The reticulocyte counts at birth between the groups after a varying number of IUTs were tested with a Kruskal-Wallis test. The secondary outcomes of proportion of neonates requiring ET and RBC transfusion after birth were tested with a $\chi 2$ test after categorising the infants in two groups: with 1-2 IUTs and >2 IUTs. Statistical analyses were performed using IBM SPSS Statistics (version 25.0; SPSS Inc, Chicago, IL).

Results

During the studied period, 250 infants treated with IUT(s) for HDFN were born and admitted to the neonatal intensive care unit of the LUMC (Figure 1). HDFN was caused by D alloimmunisation (isolated or in combination with other antibodies) in 190 infants, and by K alloimmunisation in 46 infants. One infant with D alloimmunisation was excluded due to a single unsuccessful IUT (<5 mL transfused, followed immediately by premature birth at 35 weeks of gestation). In total, 14 infants were excluded because of alloimmunisation due to other alloantibodies.

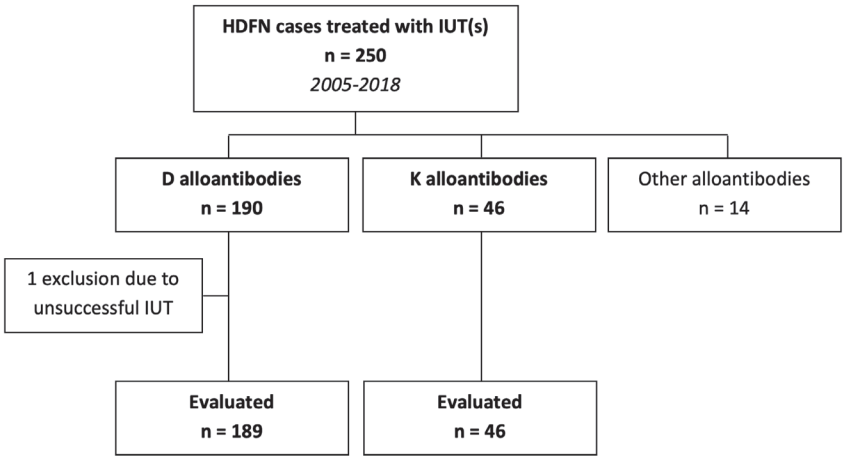


Figure 1. Flowchart of study population
HDFN, haemolytic disease of the foetus and newborn; *IUT*, intratuterine transfusion.

Baseline characteristics

Baseline characteristics of the cohort are presented in Table 1. The median gestational age at the first IUT was 29.0 weeks (IQR 24.6-32.1) in D-mediated HDFN and 25.8 weeks (IQR 22.9-28.6) in K-mediated HDFN. The median number of IUTs per foetus was 2 (IQR 2-4) and 3 (IQR 2-4).

Table 1. Baseline characteristic

Variable	Study population n = 235	D-mediated HDFN n = 189	K-mediated HDFN n = 46
Number of previous births - median (IQR)	1 (1-2)	2 (1-3)	1 (1-1)
Antenatal IVIg administration - n (%)	8 (3)	6 (3)	2 (4)
Maternal age at first IUT (years) - mean ± SD	32.0 ± 4.7	31.9 ± 4.7	32.3 ± 4.6
Gestational age at first IUT (weeks) - median (IQR)	28.0 (24.2-31.7)	29.0 (24.6-32.1)	25.8 (22.9-28.6)
Number of IUTs per foetus - median (IQR)	3 (2-4)	2 (2-4)	3 (2-4)
Maximum antibody titre - median (IQR)	256 (128-512)	256 (128-512)	128 (64-256)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *IVIg*, intravenous immunoglobulin; *n*, number; *SD*, standard deviation.

Haematologic parameters per IUT

Table 2 shows the pooled and unadjusted data of relevant haematologic parameters per consecutive IUT in D-mediated HDFN. IUTs were started at a median gestational age of 29 weeks (IQR 24.6-32.1). The median transfusion volume was 56 mL (IQR 37-80) at the first transfusion and increased with gestational age (i.e., foetal weight).

The median haemoglobin level was low before every IUT, but showed a gradual increase from the first IUT (haemoglobin 6.9 g/dL, IQR 5.3-8.5) to consecutive IUTs, as is seen in foetal haemoglobin with increasing gestational age. The median absolute reticulocyte count before the first IUT was $297 \cdot 10^9$/L (IQR 239-390) and showed an exponential decline with 70% to $92 \cdot 10^9$/L (IQR 5-176) towards the second IUT and with an additional 28% decline to $7 \cdot 10^9$/L (IQR 5-143) towards the third IUT (Figure 2). The relative reticulocyte counts showed a similar decline from 177‰ (IQR 121-242) before the first IUT to 34‰ (IQR 2-72) before the second IUT and further declined with consecutive IUTs.

The data per consecutive IUT in K-mediated HDFN are presented in Table 3. IUTs were started at a median gestational age of 26 weeks (IQR 22.9-28.6). The median transfusion volume started at 50 mL (IQR 30-67) at the first IUT.

The median absolute reticulocyte count before the first IUT was $133 \cdot 10^9$/L (IQR 29-274) and declined with 86% to $19 \cdot 10^9$/L (IQR 9-61) towards the second IUT and with an additional 8% decline to $11 \cdot 10^9$/L (IQR 5-80) towards the third IUT (Figure 2). The relative reticulocyte counts showed a similar decline from 73‰ (IQR 29-274) before the first IUT to 10‰ (IQR 4-22) beforethe second IUT and further declined with consecutive IUTs.

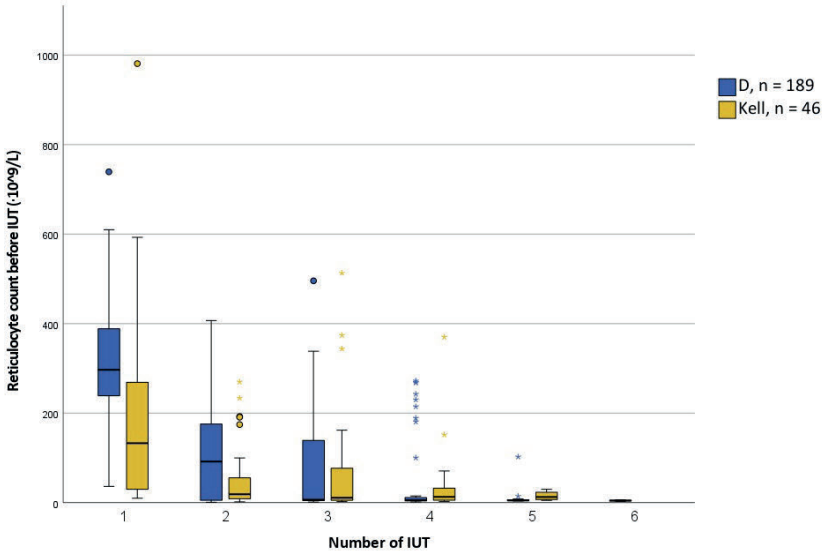


Figure 2. Boxplot of foetal reticulocyte course in D- and K-mediated HDFN
IUT, intrauterine transfusion.
Points are outliers, defined as values 1.5-3 times the interquartile range, *asterisks* are extreme outliers, defined as values >3 times the interquartile range.

Table 2. Pooled data of consecutive intrauterine transfusions in D-mediated HDFN

IUT number	1 (n = 189)	2 (n = 148)	3 (n = 89)	4 (n = 48)	5 (n = 16)	6 (n = 3)
Gestational age (weeks) - median (IQR)	29.0 (24.6-32.1)	30.0 (26.4-33.1)	31.0 (28.7-33.3)	33.0 (30.9-34.0)	33.6 (31.1-34.3)	34.1[a]
Volume transfused blood (mL) - median (IQR)	56 (37-80)[b]	63 (44-77)	74 (57-89)	77 (64-90)	77 (59-85)	94[a]
IUT administration route[c]						
Placental cord - n (%)	127 (67)	102 (69)	61 (69)	34 (71)	11 (69)	1 (33)
Intrahepatical - n (%)	2 (1)	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)
Combination - n (%)	55 (29)	41 (28)	27 (30)	12 (25)	5 (31)	2 (67)
Haemoglobin level before IUT (g/dL) - median (IQR)[d]	6.9 (5.3-8.5)	7.4 (6.1-8.9)	7.4 (6.5-8.5)	8.1 (6.8-8.9)	8.5 (7.4-9.7)	8.7[a]
Reticulocyte count before IUT (‰) - median (IQR)[e]	177 (121-242)	34 (2-72)	3 (2-56)	2 (2-4)	2 (1-2)	2[a]
Reticulocyte count before IUT ($\cdot 10^9$/L) - median (IQR[f]	297 (239-390)	92 (5-176)	7 (5-143)	5 (4-12)	5 (4-7)	5[a]

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number.
[a] No interquartile range due to n=3; [b] 2 missing values (187/189), 2 missing values (146/148), 0 missing values, 0 missing values, 0 missing values, 0 missing values; [c] groups do not add up to 100% due to 9 missing values; [d] 2 missing values (187/189), 1 missing value (147/148), 1 missing value (88/89), 0 missing values, 0 missing values, 0 missing values; [e] 12 missing values (177/189), 10 missing values (138/148), 9 missing values (80/89), 4 missing values (44/48), 1 missing value (15/16), 0 missing values; [f] 6 missing values (183/189), 2 missing values (146/148), 2 missing values (87/89), 0 missing values, 0 missing values.

Table 3. Pooled data of consecutive intrauterine transfusions in K-mediated HDFN

IUT number	1 (n = 46)	2 (n = 42)	3 (n = 32)	4 (n = 20)	5 (n = 5)
Gestational age (weeks) - median (IQR)	25.8 (22.9-28.6)	28.0 (25.3-30.9)	30.4 (28.6-32.7)	33.3 (30.8-34.3)	33.0 (32.2-34.4)
Volume transfused blood (mL) - median (IQR)	50 (30-67)	59 (40-74)	77 (66-88)	76 (70-94)	83 (73-109)
IUT administration route					
Placental cord - n (%)	28 (61)	26 (62)	21 (66)	15 (75)	4 (80)
Intrahepatical - n (%)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Combination - n (%)	16 (35)	16 (38)	11 (34)	5 (25)	1 (20)
Haemoglobin level before IUT (g/dL) - median (IQR)	6.1 (4.1-8.2)	7.4 (6.1-8.7)	7.2 (5.9-8.2)	7.6 (7.0-9.2)	7.7 (6.9-8.2)
Reticulocyte count before IUT (‰) - median (IQR)[a]	73 (29-128)	10 (4-22)	4 (2-27)	4 (2-15)	5 (3-9)
Reticulocyte count before IUT ($\cdot 10^9$/L) - median (IQR)[a]	133 (29-274)	19 (9-61)	11 (5-80)	13 (5-35)	9 (6-23)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number.
[a] 5 missing values (41/46), 3 missing values (39/42), no missing values (32/32), 1 missing value (19/20), 1 missing value (4/5).

Linear mixed models

The primary outcome was expressed as the decline (%) in absolute reticulocyte count per consecutive IUT, and was analysed by fitting a linear mixed model to account for the fact of repeated measurements and allow for covariate adjustment. The data were logarithmically transformed data (log10), the results are shown in Table 4 and 5. In D-mediated HDFN, an adjusted decline after one IUT in absolute reticulocyte count of (1-0.38) 62% (95% CI 56-67) was calculated, which is a back-calculation from the logistically transformed data reported in Table 4. After two IUTs the absolute reticulocyte count was reduced by an adjusted percentage of ($1-0.38^2$) 85% compared to the initial reticulocyte count (95% CI 81-89), by ($1-0.38^3$) 94% after three IUTs (95% CI 91-96), 99% after four IUTs (95% CI 96-99), and 100% after five IUTs (95% CI 99-100). The gestational age at the time of the first IUT per week and total number of IUTs per infant were not statistically significant in this model (p=.628 and p=.200, respectively).

In K-mediated disease, an adjusted decline after one IUT in absolute reticulocyte count of (1-0.67) 32% (95% CI 19-45) was calculated, Table 5. After two IUTs, the absolute reticulocyte count is reduced by ($1-0.67^2$) 54% compared to the initial reticulocyte count (95% CI 34-70), by ($1-0.67^3$) 70% after three IUTs (95% CI 47-83), 80% after four IUTs (95% CI 57-91), and 86% after five IUTs (95% CI 65-95). The gestational age at the time of the first IUT per week and total number of IUTs per infant were not statistically significant in this model (p=.208 and p=.196, respectively).

Table 4. Linear mixed model of the decline in reticulocyte count per consecutive IUT in D-mediated HDFN

Parameter	B	10^B	Std. error	*p*-value	95% CI	$10^{95\%\ CI}$
Intercept	2.70	501	0.51	<0.001	1.71-3.70	51-5011
Absolute reticulocyte count[a]	-0.42	0.38	0.03	<0.001	(-0.48)-(-0.36)	0.33-0.44
Gestational age at first IUT (per week)	0.01	1.02	0.01	0.628	(-0.02)-0.03	0.95-1.07
Total number of IUTs (per IUT)	-0.07	0.85	0.52	0.200	(-0.17)-0.04	0.68-1.10

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion.
[a] Log10 transformed to unskew data.

Table 5. Linear mixed model of the decline in reticulocyte count per consecutive IUT in K-mediated HDF

Parameter	B	10^B	Std. error	*p*-value	95% CI	$10^{95\%\ CI}$
Intercept	1.60	39	0.92	0.084	(-0.22)-3.41	0.60-2570
Absolute reticulocyte count[a]	-0.17	0.67	0.04	<0.001	(-0.26)-(-0.09)	0.55-0.81
Gestational age at first IUT (per week)	0.03	1.07	0.02	0.208	(-0.02)-0.08	0.95-1.20
Total number of IUTs (per IUT)	-0.13	0.75	0.10	0.196	(-0.32)-0.07	0.48-1.17

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion.
[a] Log10 transformed to unskew data.

Haematologic parameters at birth and clinical outcomes

Table 6 shows the pooled data of various haematologic parameters at birth and clinical outcomes of D-mediated HDFN. Infants were born at a median gestational age of 36 weeks, irrespective of the total amount of IUTs. The absolute and relative reticulocyte counts at birth were lower if infants received multiple IUTs, falling from $171 \cdot 10^9$/L (IQR 89-284) in infants born after one IUT to $10 \cdot 10^9$/L (IQR 3-22) in infants born after five IUTs ($p<.001$) and from 58‰ (IQR 25-83) in infants born after one IUT to 2‰ (IQR 1-5) in infants born after five IUTs. Less infants required ET after >2 IUTs (19/89, 21%), compared to infants with 1-2 IUTs (31/100, 31%), $p=.039$. Infants after >2 IUTs needed more postnatal transfusions compared to infants after 1-2 IUTs (median of 3 [IQR 2-3] vs 2 [IQR 1-3], $p=.035$). Ferritin levels increased with subsequent IUTs , from 609 µg/L after one IUT to 745 µg/L (IQR 481-2289) after four IUTs.

Table 7 shows the same data in K-mediated HDFN. These infants were also born at a median gestational age of 36 weeks. The absolute and relative reticulocyte counts at birth were lower if infants received multiple IUTs, falling from $120 \cdot 10^9$/L (IQR 46-232) in infants born after one IUT to $15 \cdot 10^9$/L (no IQR due to n=5, with 2 missing values) in infants born after five IUTs ($p=.065$) and from 35‰ (IQR 13-57) in infants born after one IUT to 3‰ (no IQR due to n=5, with 2 missing values) in infants born after five IUTs. No infants required ET after birth. Infants needed more postnatal transfusions after >2 IUTs compared with infants after 1-2 IUTs (median of 2 [IQR 1-2] vs 1 [IQR 1-1], $p<.001$). Ferritin levels increased with subsequent IUTs, from 609 µg/L (IQR 414-845) after one IUT to 776 µg/L (IQR 565-860) after four IUTs.

Table 6. Haematologic parameters at birth and clinical outcomes (infant) in D-mediated HDFN

Number of IUTs per infant	1 (n = 41)	2 (n = 59)	3 (n = 41)	4 (n = 32)	5 (n = 13)	6 (n = 3)
Male - n (%)	29 (71)	33 (56)	25 (61)	15 (47)	10 (77)	1 (33)
Gestational age at birth (weeks) - median (IQR)	36 (36-37)	36 (36-37)	36 (35-37)	36 (36-37)	36 (35-37)	37[a]
Birth weight (grams) - median (IQR)	2815 (2653-3045)	2945 (2610-3170)	2705 (2480-3001)	2860 (2590-3074)	2800 (2504-3053)	2542[a]
Haemoglobin level at birth (g/dL) - median (IQR[b]	11.9 (9.9-13.9)	12.1 (10.5-13.5)	12.9 (10.8-15.3)	12.7 (11.3-14.6)	11.4 (10.1-12.3)	12.7[a]
Reticulocytes at birth (‰) - median (IQR)[c]	58 (25-83)	43 (4-57)	13 (2-46)	3 (2-9)	2 (1-5)	2[a]
Reticulocytes at birth (·10^9/L) - median (IQR)[c]	171 (89-284)	150 (16-246)	71 (12-228)	15 (10-39)	10 (3-22)	10[a]
Ferritin level at birth (µg/L) - median (IQR)[d]	609 (414-845)	668 (564-858)	836 (592-1187)	736 (595-962)	745 (481-2289)	940[a]
Bilirubin level at birth (mg/dL) - median (IQR)	124 (83-146)	95 (70-122)	112 (82-142)	87 (70-108)	96 (81-120)	96[a]
Phototherapy (days) - median (IQR)	5 (4-6)	5 (4-5)	4 (3-5)	4 (3-5)	4 (3-5)	4[a]
Infants requiring ET - n (%)	16 (39)	15 (25)	9 (22)	6 (19)	1 (8)	3 (100)
Infants requiring RBC transfusion - n (%)[e]	36 (90)	51 (86)	37 (93)	29 (91)	13 (100)	2 (100)
Number of RBC transfusion(s) - median (IQR)[f]	2 (2-3)	2 (1-3)	2 (2-3)	3 (2-3)	3 (2-4)	4[a]

HDFN, haemolytic disease of the foetus and newborn; *ET*, exchange transfusion; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *RBC*, red blood cell.

[a] No interquartile range due to n=3; [b] 0 missing values, 1 missing value (58/59), 0 missing values, 0 missing values, 0 missing values, 0 missing values; [c] 8 missing values (33/41), 4 missing values (55/59), 6 missing values (35/41), 3 missing values (29/32), 2 missing values (11/13), 0 missing values; [d] 9 missing values (32/41), 14 missing values (45/59), 11 missing values (30/41), 5 missing values (27/32), 6 missing values (7/13), 0 missing values; [e] 1 missing value (40/41), 0 missing values, 1 missing value, (40/41), 0 missing values, 0 missing values, 1 missing value (2/3); [f] 4 missing values (37/41), 6 missing values (53/59), 4 missing values (37/41), 2 missing values (30/32), 0 missing values, 1 missing value (2/3).

Table 7. Haematologic parameters at birth and clinical outcomes (infant) in K-mediated HDFN

Number of IUTs per infant	1 (n = 4)	2 (n = 10)	3 (n = 12)	4 (n = 15)	5 (n = 5)
Male - n (%)	2 (50)	4 (40)	7 (58)	12 (80)	2 (40)
Gestational age at birth (weeks) - median (IQR)	36 (36-37)	36 (35-37)	36 (36-37)	37 (36-37)	36 (35-37)
Birth weight (grams) - median (IQR)	2867 (2493-3256)	2810 (2566-3120)	3053 (2736-3340)	3230 (2935-3500)	2890 (2385-3041)
Haemoglobin level at birth (g/dL) - median (IQR)	11.4 (11.2-15.6)	13.5 (11.9-15.5)	12.8 (11.8-14.5)	13.2 (11.1-14.2)	10.6 (7.5-12.7)
Reticulocytes at birth (‰) - median (IQR)	35 (13-57)	15 (10-43)	18 (7-38)	7 (4-41)	3[a]
Reticulocytes at birth (·10^9/L) - median (IQR)	120 (46-232)	68 (45-154)	76 (34-162)	24 (14-94)	15[a]
Ferritin level at birth (µg/L) - median (IQR)	609[b]	668 (564-858)[c]	681 (547-1248)[d]	776 (565-860)[e]	1011[f]
Bilirubin level at birth (mg/dL) - median (IQR)	56 (45-63)	64 (39-88)	61 (53-77)	74 (51-85)	60 (45-69)
Phototherapy (days) - median (IQR)	2 (1-2)	2 (2-5)	3 (2-3)	2 (2-4)	2 (2-4)
Infants requiring ET - n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Infants requiring RBC transfusion - n (%)	1 (25)	7 (70)	8 (67)	13 (87)	4 (80)
Number of RBC transfusion(s) - median (IQR)	1[g]	1 (1-1)	1 (1-2)	2 (1-2)	3 (2-4)

HDFN, haemolytic disease of the foetus and newborn; *ET*, exchange transfusion; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *RBC*, red blood cell.

[a] No interquartile range due to n=5, with 2 missing values (3/5); [b] No interquartile range due to n=4, with 2 missing values (2/4); [c] 4 missing values (6/10); [d] 5 missing values (7/12); [e] 4 missing values (11/15); [f] No interquartile range due to n=5, with 4 missing values (1/5); [g] No interquartile range due to n=1, with 3 missing values.

Discussion

Principal findings

In this study, we assessed the suppressive effect of one or multiple IUTs on the compensatory foetal erythropoiesis in severe HDFN. The foetal reticulocyte count showed an exponential decline over the course of consecutive IUTs in both D- and K-mediated HDFN, with near disappearance of foetal reticulocytes after two IUTs. This suppressive effect was seen regardless of type of alloimmunisation and has important clinical consequences for infants after birth. The exponential decrease in foetal reticulocyte counts and prolonged suppression of erythropoiesis leads to prolonged postnatal anaemia and an increased requirement of RBC transfusions after birth. A previous study performed in our centre already identified a low reticulocyte count at birth as a potential risk factor for postnatal RBC transfusions in this population[7], which we now confirmed as directly related to the number of IUTs. In addition, since infants born after multiple IUTs have less erythrocyte production and more donor blood in their circulation, the haemolysis is reduced, resulting in a lower requirement of exchange transfusions in D-mediated HDFN. No infants with K antibodies required exchange transfusion after birth in line with previous findings.[12] The strong suppressive effect was limited to the erythropoiesis, as a similar decline was not observed in foetal leukocytes and platelets.

Results

Interpretation of the reticulocyte counts and haemoglobin levels as found in this study is complicated by the lack of validated foetal reference values in unaffected pregnancies. However, Nicolaides et al.[13] described a linear physiologic decrease in absolute reticulocyte count from a mean of $27.5 \cdot 10^9$/L (or 100‰) at 17 weeks' gestation to $17.5 \cdot 10^9$/L (or 40‰) at 40 weeks' gestation. The haemoglobin concentration was described to increase linearly with gestation from respective means of 11.0 g/dL to 15.5 g/dL at 40 weeks. For our data, this means that before treatment with a first IUT, affected and severely anaemic foetuses showed, as expected, a marked reticulocytosis before IUT in line with the ongoing process of haemolysis and compensation by extramedullary haematopoiesis. The initial reticulocytosis is less pronounced in K immunisation compared with D (median reticulocyte count before IUT $133 \cdot 10^9$/L [IQR 29-274] vs $297 \cdot 10^9$/L, [IQR 239-390]), although a similar exponential decrease is observed after the course of multiple IUTs. In our data, IUTs were necessary at an earlier gestational age (25.8 vs 29.0 weeks) in K-mediated disease, but similar differences in the degree of reticulocytosis were also found in foetal blood samples that were matched for gestational age.[9]

After two IUTs, the reticulocyte count can be considered as below the physiologic reticulocyte count for gestational age regardless of type of alloimmunisation. The reticulocyte count at birth remains high for infants born after few IUTs and haemoglobin levels at birth are on the lower end of normal reference values regardless of the number of IUTs.

Interpretation

IUTs have previously been postulated to suppress erythropoiesis, although the pathophysiologic mechanism of this process is unclear. It is known that foetal erythrocytes have a shorter life-span than adult erythrocytes.[14] It may be that an IUT with adult longer living erythrocytes directly corrects anaemia to such extent that the hypoxic stimulus that leads to production of erythropoietin (EPO) is reduced. However, the ongoing haemolysis and physiologically declining effect of the transfusion should again impel compensatory erythropoiesis. IUTs may additionally disrupt foetal erythropoiesis due to the transfusion of adult haemoglobin, which has other oxygen dissociation characteristics. Foetal RBCs predominantly contain foetal haemoglobin. The concentration of foetal haemoglobin is gradually replaced by adult haemoglobin towards the end of pregnancy. At birth, foetal haemoglobin comprises 60-80% of total haemoglobin in the full-term newborn.[15] Foetal haemoglobin has a greater oxygen affinity with a left-shifted oxygen dissociation curve compared with adult haemoglobin to account for the relatively hypoxic intrauterine environment.[16] Experiments in sheep showed that exchange transfusion in sheep foetuses using adult sheep blood resulted in an overall decrease in oxygen affinity and saturation and, interestingly in view of our results, an increased reticulocytosis, whereas haemoglobin levels remained constant.[17] Adult haemoglobin is, however, also known to provide better peripheral tissue oxygenation compared with foetal haemoglobin[16], which may result in a local reduction of hypoxic stimulus, causing reduced EPO production, which might explain the observed drastic decline in reticulocytes. In our institute, foetal EPO levels are not routinely measured. If an EPO level decline indeed underlies the found reticulocyte decline, it may be useful to start EPO treatment before birth.

Interestingly, we found a similar disruption of (compensatory) foetal erythropoiesis by IUTs in foetuses and infants with D- and K-mediated HDFN, whereas these are known to have a different pathophysiology and clinical course. Even low antibody titres in pregnancy can cause severe foetal anaemia in K alloimmunisation[18], for example, although these neonates require overall less phototherapy and less exchange transfusions compared with D alloimmunisation. There is in both D and K alloimmunisation a similar high degree of neonatal anaemia and transfusion dependency after birth.[12] K antigens appear on erythroid progenitor cells early

in erythropoiesis[19], and erythroid suppression seems to be the predominant mechanism in producing foetal anaemia, rather than haemolysis.[9-11] This is reflected by our finding that the reticulocyte count before the first IUT was substantially lower in K than in D immunised pregnancies (133 vs 297·10^9/L). The difference cannot be explained by the difference in gestational age only. As erythroid suppression is already part of the pathogenesis in K alloimmunisation, it is of particular interest that one or multiple IUTs have an added suppressive effect.

We also considered the alternative hypothesis that the disrupted erythropoiesis is related to iron load. Iron deficiency can cause and prolong anaemia, but excessive iron as caused by the ongoing haemolysis and the multiple IUTs, can also be toxic to erythropoiesis.[20,21] Despite overall high ferritin levels at birth in this transfused population, these levels are not high enough to cause toxicity or explain the degree of erythropoiesis suppression observed in this study.

Research implications

Our study has several research implications for the future. More studies are needed to investigate the relationship between IUTs, HDFN, and EPO to further understand these observations. At our centre we are currently performing a randomised clinical trial to assess the effect of exogenous administered darbepoetin alfa after birth on postnatal transfusion dependency in IUT-treated infants (NCT03104426), of which the first results are expected in 2022.

The clinical implications of the neonatal reticulocyte count after birth were elaborated on in previous work of our research group, which identified a low reticulocyte count after birth as predictor of postnatal RBC transfusion need.[7] We recommend postnatal measurement of reticulocyte count along with postnatal haemoglobin for a period of two to three months as part of neonatal follow-up after birth to shed further light on the state of recovery of the erythropoiesis.

Strengths

The major strength in this study is inclusion of a large group of infants treated with the same protocol in one centre of expertise, resulting in a near-complete collection of data of an increasingly rare disease. This enabled us to further detail the haematologic effects of IUTs and HDFN and further unravel its pathophysiology. Ultimately, we are moving forward toward further individualisation of treatment and follow-up of infants affected by HDFN, identifying

those groups of infants at the greatest risk for a complicated disease course and pinpointing treatment towards these infants.

Limitations

One of the limitations of this study is that there are no foetal blood samples in between IUTs. It is expected that the reticulocyte course has a more fluctuating course after an IUT is administered than what can be seen based on the available samples. As mentioned previously, the lack of endogenous foetal and neonatal EPO levels is also of concern and could yield additional crucial information in future studies as well as follow-up of neonatal antibody titres. Due to the nature of the IUT procedure, missing values were to be expected and are reported with the data. The small volume blood samples are susceptible to agglutination, which may be enhanced by improper handling of the sampled volume after the procedure (turning of sample tube). These missing values are however considered as “at random” and no further statistical measures were taken to address this. Finally, reticulocyte counts have to be seen as so-called endogenous variables, i.e., counts at a given time point depend on values observed at previous time points as they influence the clinical decision of administering IUTs. More complex interactions between IUTs and reticulocyte counts than discussed here are thinkable. We believe that our interpretation is the clinically most plausible one.

Conclusions

From a pathophysiologic and clinical point of view, our study highlights the potential negative effect of one or multiple IUTs on erythropoiesis and the observed prolonged effects after birth. A distinction can be made between infants treated with one or two IUTs and infants treated with multiple IUTs. The latter group not only reflects more severe disease as indicated by the severe foetal anaemia prompting the higher amount of IUTs, but has an additional more pronounced suppression of erythropoiesis. In conclusion, we state that after IUT treatment for HDFN, an exponential decrease in foetal reticulocyte counts is observed and infants born after multiple IUTs show a prolonged suppressed erythropoiesis with a greater transfusion need.

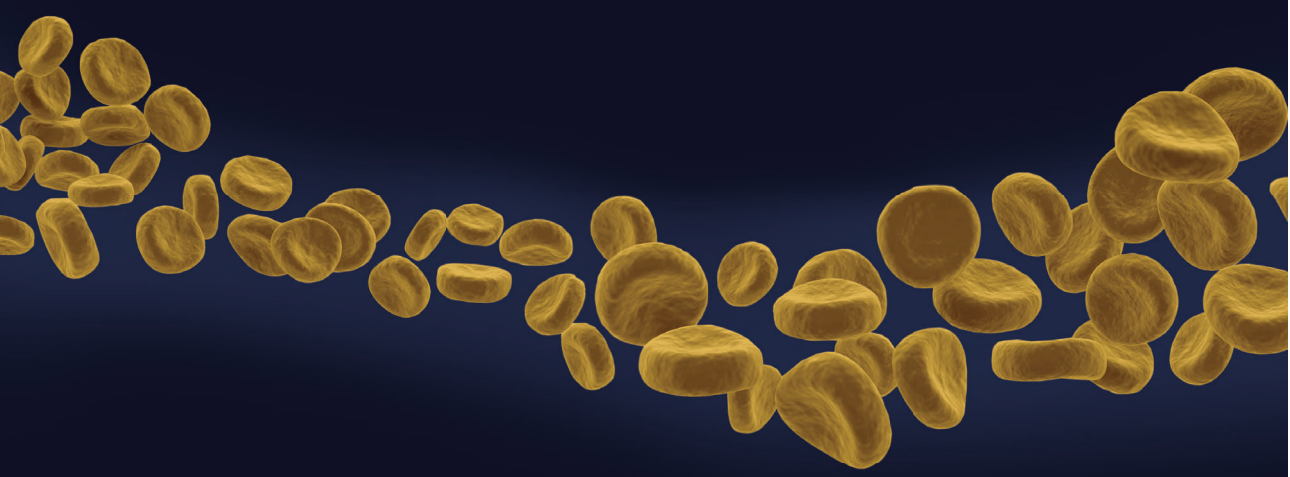
References

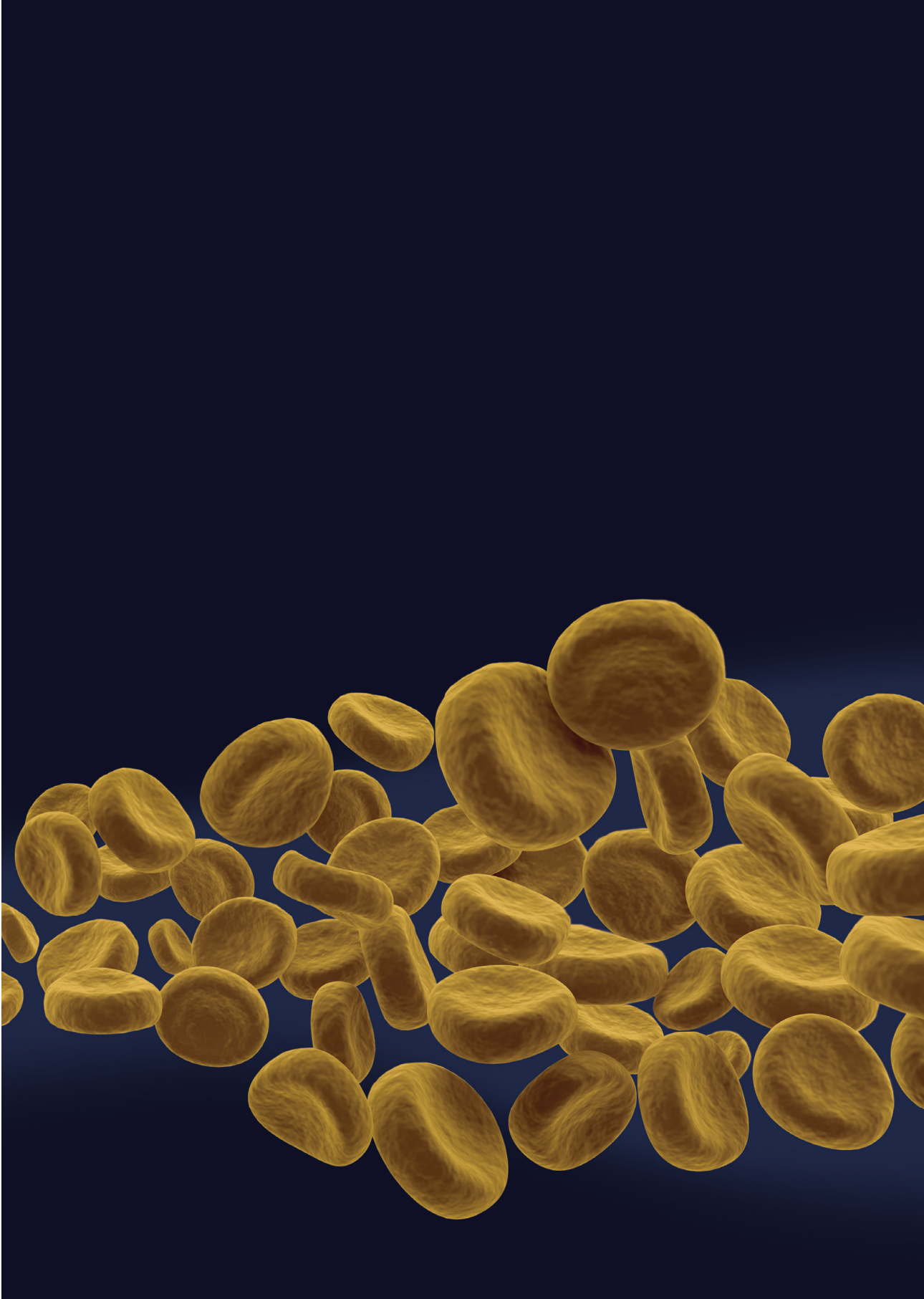
1. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Hemolytic disease of the fetus and newborn. Vox Sang 2015;109(2):99-113.
2. Urbaniak SJ, Greiss MA. RhD hemolytic disease of the fetus and the newborn. Blood Rev 2000;14(1):44-61.
3. Millard DD, Gidding SS, Socol ML, MacGregor SN, Dooley SL, Ney JA, Stockman JA. Effects of intravascular, intrauterine transfusion on prenatal and postnatal hemolysis and erythropoiesis in severe fetal isoimmunization. J Pediatr 1990;117(3):447-454.
4. Goodrum LA, Saade GR, Belfort MA, Carpenter RJ, Jr., Moise KJ, Jr. The effect of intrauterine transfusion on fetal bilirubin in red cell alloimmunization. Obstet Gynecol 1997;89(1):57-60.
5. Vietor HE, Klumper F, Meerman RJ, Brand A, Kanhai HH. Intrauterine transfusions influence fetal leukocyte counts and subsets. Prenat Diagn 1998;18(4):325-331.
6. De Boer IP, Zeestraten EC, Lopriore E, van Kamp IL, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008;198(1):54 e51-54.
7. Ree IMC, de Haas M, Middelburg RA, Zwiers C, Oepkes D, van der Bom JG, Lopriore E. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth. Br J Haematol 2019;186(4):565-573.
8. van Kamp IL, Klumper FJ, Meerman RH, Oepkes D, Scherjon SA, Kanhai HH. Treatment of fetal anemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. Acta Obstet Gynecol Scand 2004;83(8):731-737.
9. Vaughan JI, Warwick R, Letsky E, Nicolini U, Rodeck CH, Fisk NM. Erythropoietic suppression in fetal anemia because of Kell alloimmunization. Am J Obstet Gyneco. 1994;171(1):247-252.
10. Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anemia. N Engl J Med 1998;338(12):798-803.
11. Daniels G, Hadley A, Green CA. Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion 2003;43(1):115-116.
12. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, van Kamp IL, Oepkes O, Wlather FJ, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100(3):312-316.
13. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell isoimmunisation. Lancet 1988;1(8594):1073-1075.
14. Pearson HA. Life-span of the fetal red blood cell. J Pediatr 1967;70(2):166-171.
15. Bard H. The postnatal decline of hemoglobin F synthesis in normal full-term infants. J Clin Invest 1975;55(2):395-398.
16. Finne PH, Halvorsen S. Regulation of erythropoiesis in the fetus and newborn. Arch Dis Child 1972;47(255):683-687.
17. Battaglia FC, Bowes W, McGaughey HR, Makowski EL, Meschia G. The effect of fetal exchange transfusions with adult blood upon fetal oxygenation. Pediatr Res 1969;3(1):60-65.
18. Slootweg YM, Lindenburg IT, Koelewijn JM, Van Kamp IL, Oepkes D, De Haas M. Predicting anti-Kell-mediated hemolytic disease of the fetus and newborn: diagnostic accuracy of laboratory management. Am J Obstet Gynecol 2018;219(4):393 e391-393 e398.
19. Southcott MJ, Tanner MJ, Anstee DJ. The expression of human blood group antigens during erythropoiesis in a cell culture system. Blood 1999;93(12):4425-4435.

20. Berger HM, Lindeman JH, van Zoeren-Grobben D, Houdkamp E, Schrijver J, Kanhai HH. Iron overload, free radical damage, and rhesus haemolytic disease. Lancet 1990;335(8695):933-936.

21. Isidori A, Borin L, Elli E, Latagliata R, Martino B, Palumbo G, Pilo F, et al. Iron toxicity - Its effect on the bone marrow. Blood Rev 2018;32(6):473-479.

Part 3 - Neonatal management and complications





Chapter four

Exchange transfusions in severe Rh-mediated alloimmune haemolytic disease of the foetus and newborn: a 20-year overview on the incidence, associated risks and outcome

Isabelle M.C. Ree
Carolin F.J. Besuden
Vivianne E.H.J. Wintjens
Joanne (E.)J.T. Verweij
Dick Oepkes
Masja de Haas
Enrico Lopriore

Vox Sanguinis 2021;116(9):990-997.

Abstract

Background and objectives

Guidelines and indications for exchange transfusion in haemolytic disease of the foetus and newborn (HDFN) have changed drastically in the past decades, causing a decline in exchange transfusion rate. This study aims to evaluate the incidence of exchange transfusions (ETs) in neonates with Rh-mediated HDFN over the past 20 years at our centre, and report potential ET-related complications as well as indicators for bilirubin encephalopathy.

Material and methods

In this observational study, 438 neonates were included with HDFN, born ≥35 weeks' gestation at the Leiden University Medical Centre between January 2000 and July 2020. The incidence of ET and procedure-related complications were assessed in three consecutive time periods determined by changes in guidelines and indications for ET.

Results

The incidence of ET in our centre declined from (104/156) 67% (time period 2000-2005), to (39/181) 22% (2006-2015) and to (10/101) 10% (2015-2020, $p<.001$). The maximum bilirubin levels in neonates after birth increased from 13.6 mg/dL (or 233 μmol/L), to 15.0 mg/dL (257 μmol/L) and to 15.3 mg/dL (263 μmol/L). The incidence of complications associated with the use of ET (including sepsis, haematologic disorders and respiratory failure) remained stable throughout the years, and no neonates died during the study period.

Conclusion

ET incidence declined significantly over the past two decades. Decrease in ET incidence, and concomitant decrease in exposure and expertise, was not associated with an increase in procedure-related complications.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is caused by an incompatibility between maternal and foetal red blood cell (RBC) antigens. Destruction of the foetal red RBCs by maternal alloantibodies results in foetal and neonatal haemolytic anaemia, which can be treated antenatally with intrauterine transfusions (IUTs) and postnatally with RBC transfusions. Haemolysis may also lead to hyperbilirubinaemia and can result in acute and chronic bilirubin encephalopathy.

Hyperbilirubinaemia is treated with intensive phototherapy and exchange transfusion (ET). ET is recommended for infants whose bilirubin levels continue to rise to exchange levels, despite intensive phototherapy.[1] ET removes excess bilirubin from the neonatal blood circulation as well as maternal antibodies and antibody-coated erythrocytes.[1-3] Approximately 85% of the neonatal blood is replaced by irradiated donor blood by double-volume exchange transfusion.[3,4] While being effective in the acute treatment of hyperbilirubinaemia, ET is an invasive procedure requiring central lines and has potentially severe side effects. Mortality rates around 0.3% are reported in term neonates, but increase above 10% in preterm neonates.[4-6] Morbidity rates are reported up to 24%, and include cardiorespiratory instability, catheter-related complications, thrombocytopenia and sepsis.[7-9]

Data on changes in the incidence and complications of ET in severe HDFN are limited. In our centre, we noticed a reduction of the need for ET in the past decades, partly related to changes in our guidelines and indications for ET, which have become more restrictive over the years. Whether a reduction in exposure and expertise in performing an invasive and complex procedure as an ET is also associated with an increased risk of complications, is not well known.

The aim of this study is to give an overview of the incidence of exchange transfusions in neonates with severe Rh-mediated HDFN over the past 20 years at our centre, and to report potential ET-related complications, as well as indicators for bilirubin encephalopathy.

Methods

Study design and population

This is an observational cohort study conducted at the Leiden University Medical Centre (LUMC), the Dutch national referral centre for severe HDFN and foetal therapy. In the Netherlands, all pregnant women with RhD immunisation and an ADCC (antibody-dependent cell-mediated cytotoxicity) of >50% and/or antibody titre ≥16, as well as with Rh immunisation other than D and ADCC >30% and/or antibody titre ≥16, are referred to the LUMC.[10] Subsequently, these high-risk pregnancies are monitored by serial Doppler measurements to assess the velocity of the blood flow in the middle cerebral artery. If this velocity exceeds 1.5 multiples of the median or if signs of hydrops are present, treatment with IUT is indicated. IUTs can be administered until 34-35 weeks' gestation, after which induced delivery is preferred to IUT treatment. The IUT technique used in the Netherlands has been previously described.[11]

All (near-) term neonates (≥35 weeks' gestation) with HDFN due to maternal red cell alloimmunisation against Rh antigens (D, C, c, Cw, E and e) admitted to the LUMC between 1 January 2000 and 31 June 2020 were eligible for this study.

Neonates with blood group alloimmunisation caused by non-Rh antigens were excluded as these antigens show different pathophysiological characteristics and great variation in exchange transfusion risk after birth.[12] Neonates born <35 weeks' gestation were excluded as prematurity itself is a major risk factor for hyperbilirubinaemia and ET treatment and is associated with greater odds of death following ET compared to term infants.[13] In addition, neonates who received intravenous immunoglobulins (IVIgs, n=41) as part of a randomised controlled trial (RCT, LIVIN trial, identifier ISRCTN14013064) between 2006 and 2010, were excluded as IVIg is not a standard practice at the LUMC.

The study cohort was divided in three different time periods according to changes in ET guidelines:

Group I: 1 January 2000 to 31 December 2005

In this first group, the bilirubin threshold for ET was a total serum bilirubin level at birth >3.5 mg/dL (measured in umbilical cord blood, or, more often, in neonatal blood at birth) and/or a rise of bilirubin >0.5 mg/dL/h despite intensive phototherapy.[14] In all neonates, bilirubin levels were measured every 2-3 hours according to protocol during the first few days after birth. The differences in bilirubin thresholds have been previously described.[9]

Group II: 1 January 2006 to 31 March 2015

On 1 January 2006, we implemented new, more restrictive, ET guidelines based on the updated guideline of the American Academy of Pediatrics (AAP).[2] After the guideline change, the new criteria for ET were: (1) total serum bilirubin above thresholds according to the AAP guideline, and/or (2) rise of bilirubin >0.5 ml/dL/h despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level.[9]

Group III: 1 April 2015 to 31 June 2020

This third group encloses the years after the most recent guideline change at our centre, implemented in April 2015. The AAP guideline advises to classify proven blood group alloimmunisation as 'high risk' for the rise of bilirubin to levels that might cause bilirubin encephalopathy.[2] After a consensus meeting at our centre, we decided not to categorise proven blood group antagonism as an extra risk factor as the blood-brain barrier does not decrease in function with the presence thereof. Neonates with proven blood antagonism born after April 2015 were therefore categorised as 'standard risk' when choosing which threshold curve (low, standard or high risk) to use for phototherapy and ET.

Throughout the study period, ET were performed with blood exchange volumes of 100-200 ml/kg. The irradiated blood product consists of leukocyte-reduced erythrocytes and plasma of two donors, less than 5 days old with a haematocrit of 0.50-0.65 g/L. No albumin or calcium infusions were given prior or during ET.

Outcome measures

The primary outcome was the incidence of ETs. Secondary outcomes included the timing of the first ET in hours, the duration of phototherapy in days, postnatal RBC transfusion dependency, length of stay at the neonatal intensive care unit (NICU) and ET-related complications.

Data collection

The following obstetric and neonatal data were directly recorded at our centre and obtained from patient's medical files: foetal haemoglobin at first IUT and number of IUTs, gestational age and weight at birth, gender, mode of delivery, haemoglobin and bilirubin level at birth (conjugated and unconjugated), type of alloimmunisation, maximum bilirubin level during admission, number of ETs and timing of ETs, postnatal RBC transfusions (not including RBCs given as part of ET), respiratory distress (defined as need for mechanical ventilation), umbilical vein catheterisation, number of days of phototherapy before discharge home or transfer to

another hospital, results from standard cerebral ultrasound (intraventricular haemorrhage graded according to the grading system of Papile[15] and periventricular leukomalacia (PVL) according to de Vries et al.[16]), results from standard hearing screening and total length of hospital stay. The following complications of ET were recorded: proven sepsis (defined as clinical symptoms of infection combined with positive blood culture after the moment of ET), thrombosis (defined as the detection of a vascular thrombosis on ultrasound examination), mechanical ventilation, leukopenia (defined as leukocytes $<5{\cdot}10^9$/L), thrombocytopenia (defined as platelets $<100{\cdot}10^9$/L), platelet transfusion rate, hypocalcaemia (defined as calcium <8.0 mg/dL), hyperkalaemia (defined as potassium >6.5 mEq/L) and neonatal mortality.[17] Follow-up data on RBC transfusions after discharge from our centre were collected from referral hospitals with written consent from parents or caregivers. RBC transfusions were administered in term neonates with HDFN when haemoglobin levels fall below 10.5 g/dL for day 0-6, below 8.9 g/dL for day 7-13, and below 7.2 g/dL from day 14 onwards. Before February 2014, haemoglobin thresholds for transfusion were 9.6 g/dL for day 7-13 and 8.0 g/dL from day 14 onwards. A transfusion of 15 ml/kg irradiated packed erythrocytes less than 5 days old was advised throughout the study period, with a haematocrit of 0.50-0.65 g/L.

Statistical analysis

Data was reported as means and standard deviations (SD), or as medians and interquartile range (IQR), when appropriate. The primary outcome was tested by χ^2 test. Statistical analysis was performed using IBM SPSS Statistics (version 26.0; SPSS Inc, Chicago, IL).

Ethical considerations

Due to the non-invasive nature of this study, a waiver of consent was granted by the medical ethics committee of our centre.

Results

During the study period of 20 years, 612 neonates with severe HDFN were admitted to the NICU of the LUMC, and 438 neonates met the inclusion criteria and were eligible for this study. We excluded 87 neonates due to HDFN primarily caused by non-Rh antibodies, 56 neonates with a gestational age <35 weeks, 10 neonates who fulfilled both exclusion criteria and 41 neonates treated with IVIg as part of the LIVIN trial (Figure 1).

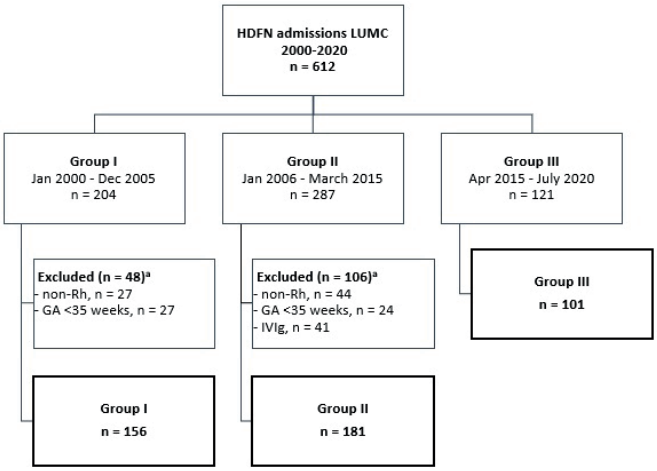


Figure 1. Flowchart of study participants
GA, gestational age; *HDFN*, haemolytic disease of the foetus and newborn; *IVIg*, intravenous immunoglobulin; *LUMC*, Leiden University Medical Centre; *n*, number.
[a] Some neonates meet more than one exclusion criteria; hence, combined numbers per criterion can exceed the total number of exclusions.

Baseline characteristics for each of the three cohorts are shown in Table 1. RhD was the most common causative Rh antigen of HDFN in all three groups (89%, 85% and 92%, respectively), followed by Rhc. The occurrence of multiple Rh alloantibodies was 51% in group I, 43% in group II and 39% in group III. The occurrence of a non-Rh alloantibody, besides a primary Rh alloantibody, was, respectively, 7% in group I, 6% in group II and 3% in group III. This most often involved alloantibodies against K or Jka antigens.

Table 1. Baseline characteristics

	Group I (n = 156)	Group II (n = 181)	Group III (n = 101)
Neonates treated with IUT(s) - n (%)	99 (63)	102 (56)	61 (60)
Number of IUT(s) per neonate[a]	3 (2-4)	2 (2-4)	2 (1-3)
Gestational age at birth - weeks[a]	37 (36-37)	37 (36-37)	37 (36-37)
Birth weight - grams[b]	2972 ± 443	2987 ± 470	2913 ± 348
Caesarean delivery - n (%)	48 (31)	41 (23)	27 (27)
Male gender - n (%)	87 (56)	114 (63)	47 (47)
Primary type of alloantibodies			
Rh D - n (%)	139 (89)	153 (85)	93 (92)
Rh C - n (%)	1 (1)	2 (1)	0 (0)
Rh c - n (%)	13 (8)	17 (9)	8 (8)
Rh Cw - n (%)	0 (0)	2 (1)	0 (0)
Rh E - n (%)	3 (2)	7 (4)	0 (0)
Additional non-Rh alloantibodies - n (%)	11 (7)	10 (6)	3 (3)
Haemoglobin level at birth - g/dL[b,c]	11.6 ± 2.6	13.4 ± 3	13.5 ± 2.6
Unconjugated bilirubin level at birth - mg/dL[b,d]	5.5 ± 2.8	5.7 ± 2.9	5.1 ± 2.3
Unconjugated bilirubin level at birth - µmol/L[b,d]	94 ± 48	97 ± 49	87 ± 39
Conjugated bilirubin level at birth - mg/dL[a,e]	0.5 (0.4-0.8)	0.6 (0.5-0.8)	0.5 (0.4-0.6)
Conjugated bilirubin level at birth - µmol/L[a,e]	9 (6-14)	10 (8-13)	8 (6-11)

IQR, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *SD*, standard deviation.
[a] Median (*IQR*); [b] mean ± *SD*; [c] 0 missing values in group I, 5 missing values group II, 1 missing value group III; [d] 1 missing value group I, 5 missing values group II, 1 missing value group III; [e] 17 missing values group I, 35 missing values group II, 5 missing values group III.

Neonatal treatment data and outcome measures are presented in Table 2. The ET incidence decreased significantly from 104/156 (67%) in group I (before the AAP guideline change), to 39/181 (22%) in group II (after the AAP guideline change) and was further reduced since then to 10/101 (10%) in the most recent group III (p<.001). Numbers of ET per year are shown in Figure 2.

In group I, 106/156 (69%) neonates were treated with postnatal RBC transfusion(s), 37/156 (81%) in group II, and 68/101 (74%) neonates in group III. The maximum bilirubin reached levels >25.0 mg/dL in 8 neonates: 1 (1%) in group I, 6 (3%) in group II and 1 (1%) in group III. In group I, 129/156 (83%) neonates had an umbilical venous catheter, 68/156 (38%) in group II and 39/101 (39%) in group III (Table 2).

Table 2. Neonatal outcomes

	Group I (n = 156)	Group II (n = 181)	Group III (n = 101)
Neonates treated with ET(s) - n (%)[a]	104 (67)	39 (22)	10 (10)
Maximum unconjugated bilirubin level - mg/dL[b]	13.6 ± 4.7	15.0 ± 5.2	15.3 ± 4.7
Maximum unconjugated bilirubin level - µmol/L[b]	233 ± 80	257 ± 89	262 ± 80
Bilirubin > 25.0 mg/dL - n (%)[c]	1 (1)	6 (3)	1 (1)
Umbilical venous catheter - n (%)	129 (83)	68 (38)	39 (39)
Duration of phototherapy - days[d,e]	4 (3-5)	5 (3-6)	5 (4-6)
Neonates receiving RBC transfusion(s) - n (%)[f]	106 (69)	137 (81)	68 (74)
Number of RBC transfusions per neonate[d,g]	2 (1-3)	2 (1-3)	2 (1-3)
Mechanical ventilation - n (%)	6 (4)	1 (1)	1 (1)
Proven sepsis - n (%)[h]	6 (4)	6 (4)	5 (5)
Duration of NICU admission - days	6 ± 3	7 ± 3	7 ± 2
Mortality - n (%)	0 (0)	0 (0)	0 (0)

ET, exchange transfusion; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *NICU*, neonatal intensive care unit; *RBC*, red blood cell; *SD*, standard deviation.
[a] *p*-value <.001; [b] mean ± SD; [c] absolute "medical emergency" value indicating direct need for intensive phototherapy as recommended by the *AAP*,[2] equal to 428 µmol/l; [d] median (*IQR*); [e] 22 missing values group I, 1 missing value group II, 0 missing values group III; [f] 2 missing values group I, 12 missing values group II, 9 missing values group III; [g] 2 missing values in group I, 3 missing values group II, 6 missing values group III; [h] 0 missing values in group I, 8 missing values in group II, 1 missing value in group III.

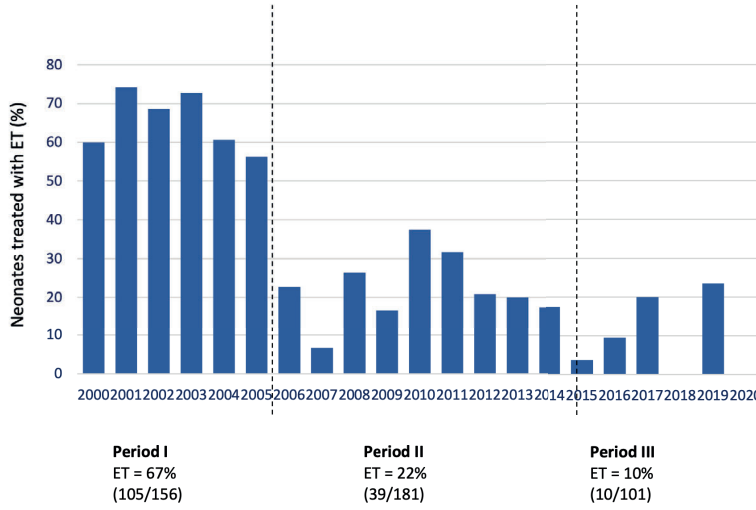


Figure 2. Incidence of exchange transfusion throughout the years
ET, exchange transfusion.

Characteristics and complications in subgroup of neonates treated with ET are presented in Table 3. The number of ETs per neonate did not differ between groups (median 1 ET per neonate); the time after birth before an ET was performed increased from a median of 6 hours in group I to a median of 50 hours in group III.

In group I, four neonates required mechanical ventilation in relation to ET treatment, none in the other groups. Sepsis after the first or following ETs occurred in 6% of group I, respectively, 11% and 10% in groups II and III. All neonates received the ET through an umbilical vein catheter. Umbilical vein thrombosis was diagnosed in one case, which was detected after an ultrasound examination was performed due to persisting thrombocytopenia. Leukocytopenia occurred in 63% of the cases in group I, 71% in group II and 33% in group III. Thrombocytopenia $<100 \cdot 10^9$/L occurred in almost all neonates that underwent at least one ET, but severe thrombocytopenia $<25 \cdot 10^9$/L was rare (21 neonates (20%) in group I, five neonates (13%) in group II and none in group III). The occurrence of hypocalcaemia <8.0 mg/dL after ET was 12% in group I, 15% in group II and did not occur in group III. Hyperkalaemia >6.5 mEq/L after ET occurred in only two cases, one neonate in group I and one neonate in group II.

Hearing screening was conducted in a great majority of neonates in group II and III, but only in 43 (41%) of group I. All neonates tested passed the screening. Cerebral ultrasound was conducted in a great majority of group II (90%) and group III (100%), but only in half of the

neonates (49%) of group I. Ultrasound showed one case of severe IVH (grade 3) and one case of cystic PVL in group II. No neonates died in our study population.

Table 3. Characteristics and complications in subgroup of neonates treated with ET

	Group I (n = 104)	**Group II (n = 39)**	**Group III (n = 10)**
Number of ET(s) per neonate[a]	1 (1-2)	1 (1-2)	1 (1-1)
Time to first ET - hours after birth[a]	6 (5-9)	31 (15-55)	50 (18-65)
Mechanical ventilation - n (%)	4 (4)	0 (0)	0 (0)
Proven sepsis as related to ET - n (%)[b]	6 (6)	4 (11)	1 (10)
Umbilical vein thrombosis - n (%)[c]	1 (1)	0 (0)	0 (0)
Leukocytopenia <5·10^9/L - n (%)[d]	66 (63)	27 (71)	3 (33)
Thrombocytopenia <100·10^9/L - n (%)[e]	101 (98)	36 (97)	10 (100)
25-49·10^9/L	41 (40)	19 (50)	3 (30)
<25·10^9/L	21 (20)	5 (13)	0 (0)
Neonates receiving platelet transfusion(s) - n (%)	56 (54)	23 (59)	3 (30)
Hypocalcaemia <8.0 mg/dL - n (%)	12 (12)	6 (15)	0 (0)
Hyperkalaemia >6.5 mEq/L - n (%)	1 (1)	1 (3)	0 (0)
Hearing screening performed - n (%)	43 (41)	32 (82)	8 (80)
Hearing screening passed - n (%)[f]	43 (100)	32 (100)	8 (100)
Cerebral ultrasound performed - n (%)	51 (49)	35 (90)	10 (100)
Minor IVH, grade I or II - n (%)	0 (0)	0 (0)	0 (0)
Major IVH, grade III or IV - n (%)	0 (0)	1 (3)	0 (0)
Cystic PVL - n (%)	0 (0)	1 (3)	0 (0)
Mortality - n (%)	0 (0)	0 (0)	0 (0)

ET, exchange transfusion; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *IVH*, intraventricular haemorrhage; *PVL*, periventricular leukomalacia; *SD*, standard deviation
[a] Median (IQR); [b] sepsis defined as symptomatic infection with positive blood culture, onset later than first ET; [c] only one neonate underwent a catheter ultrasound; [d] 1 missing value in group II; [e] 3 missing values, 1 in each group; [f] 61 missing values in group II, 7 missing values in group II, 2 missing values in group III.

Discussion

In this study we showed a significant decline of ET incidence in neonates with severe HDFN due to severe Rh-mediated HDFN over the past 20 years. The incidence of ET treatment declined from 67% to 10%. The time to first ET after birth was postponed from 6 to 50 hours. This impressive decline in ET can be attributed to the changes and implementation of increasingly restrictive ET guidelines as well as to improved use of intensive phototherapy. Importantly and reassuringly, the strong decline in ET incident and thus decreased exposure and expertise with this complex procedure, was not associated with an increase in procedure-related complications. In the Netherlands, this is presumably due to the centralisation and thus specialisation of care for neonates with HDFN.

Only few studies have evaluated the changes in the use of ET over the years[8,13,18-21], and although accurate comparison is not possible, these studies also show an overall sharp decline in ETs in HDFN without increase in adverse events related to ET. Comparison with other centres is complicated due to various time cohorts chosen, different study populations and definitions of (severe) HDFN vary greatly, as do local phototherapy and ET protocols and guidelines. A study from the USA compared 71 infants treated with ET between 1986 and 1995 to 36 infants treated with ET between 1996 and 2006 (overall decline of 35/71, 49%). The ET-related complications incidence was 14% vs 7% in the two cohorts (p=.270). Of the studied cases, only 41% was treated with ET due to hyperbilirubinaemia caused by Rh-mediated HDFN, and an additional 28% due to ABO incompatibility.[8] A study from Norway showed a decrease in ET incidence from (39/80) 49% in the period 1993-1998 to (12/96) 12% in the period 1999-2003.[18] The study group included infants with Rh-mediated HDFN, as well as ABO incompatibility. This decline was attributed to the introduction of standard IVIg treatment in 1998. A study in India reported a decline of ET-incidence from 6.8% of the total neonatal admissions in 2006 to 0.3% in 2016, but did not report on underlying indications for ET.[19] A recently published large multicentre cohort study assessing the prevalence of ET and ET-associated morbidity and mortality in the USA between 1997 and 2016, reported a decline in ETs of 0.3% to 0.05% among all NICU admissions and found similarly high numbers of thrombocytopenia and leukopenia. However, the study also did not report on underlying causes of hyperbilirubineamia and included neonates as young as 23 weeks of gestation, complicating accurate comparison.[13]

For an increasingly rare disease as HDFN, international research would be highly recommendable to register and address these major differences between countries and treatment centres. It is important to have seemingly simple numbers as the current ET incidence available for counselling of parents and caregivers, organisation of care and for further research in which ET incidence can be a potential outcome measure.

In our cohort, no cases of acute transfusion reactions such as transfusion-related lung injury and transfusion-associated circulatory overload were reported. The features of these reactions are presumably less outspoken and therefore under recognised in neonates.[22]

Several studies that describe the decline of ETs during the last three decades suggest that the decrease in ETs could be explained by the administration of IVIg to neonates.[18] A Cochrane review by Zwiers et al.[23] was inconclusive on this subject since the studies that showed a reduction of ETs after administration of IVIg were of low quality and of high risk of bias. The only two high-quality placebo-controlled randomised studies did not provide any evidence that administration of IVIg reduced the need for ETs.[24,25] In our centre, we do not administer IVIg (except during the study period of the aforementioned LIVIN study[24]) and therefore, the observed decline in ETs in our cohorts cannot be explained by IVIg treatment.

Earlier work by our study group showed a tendency toward an increase in the postnatal RBC transfusion rate, correlated with a decrease in ET incidence in neonates with severe HDFN. This effect was attributed to the removal of antibodies and IgG coated erythrocytes during ET, hence reducing the haemolytic process.[10,18,23] Although morbidity of RBC transfusion (reported between 0.014 and 0.04%[26]) is much less than the morbidity of ET treatment (between 7 and 24%[9]), it is still of interest that in the current study, no further increase in neonatal RBC transfusions was observed. A possible explanation could be the implementation of a more restrictive RBC transfusion threshold at our department in February 2014.

This study has several strengths and limitations. One of the major strengths is the setting of one national referral centre for severe HDFN, providing us with a homogenous and near-complete collection of data and follow-up records of an increasingly rare disease. However, the results must be carefully interpreted in the context of our study population, as it is a selection of (very) severe HDFN cases as result of the referral guidelines in the Netherlands. Neonates not treated with IUT, particularly the less severe cases that did not require foetal therapy, were probably more likely to have been admitted to other centres.

Another major limitation of this study, and of the research in the field of HDFN in general, is the lack of long-term outcome results of these neonates. Although cerebral ultrasound and hearing screening are now part of routine care after IUT treatment and ET treatment at our centre and no neonates in this study showed abnormalities in these tests, the occurrence of long-term (mild) symptoms of bilirubin encephalopathy is unknown, as well as clear, absolute bilirubin cut-off values which may give rise to such symptoms.

In conclusion, the need for ET in neonates with severe HDFN admitted to our centre has gradually decreased and has now become relatively rare. Reduction in ET incidence and therefore in expertise in performing this complex procedure was not associated with an increase of procedure-related complications. Nevertheless, if the exposure of physicians to ET treatment will continue to decline, centralisation of this procedure in specialised tertiary care centres may be necessary to maintain sufficient experience.

References

1. Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. Expert Rev Hematol 2017;10(7):607-616.
2. American Academy of Pediatrics Subcommittee on H. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114(1):297-316.
3. al-Alaiyan S, al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med 1999;27(2):112-115.
4. Smits-Wintjens VEHJ, Walther FJ, Lopriore E. Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. Semin Fetal Neonatal Med 2008;13(4):265-271.
5. Murki S, Kumar P. Blood exchange transfusion for infants with severe neonatal hyperbilirubinemia. Semin Perinatol 2011;35(3):175-184.
6. Dennery PA, Seidman DS, Stevenson DK. Neonatal hyperbilirubinemia. N Engl J Med 2001;344(8):581-590.
7. Chitty HE, Ziegler N, Savoia H, Doyle LW, Fox LM. Neonatal exchange transfusions in the 21st century: a single hospital study. J Paediatr Child Health 2013;49(10):825-832.
8. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal exchange transfusions and its effect on exchange-related morbidity and mortality. Pediatrics 2007;120(1):27-32.
9. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, Oepkes D, Walther FJ, Lopriore E. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010;99:65-70.
10. Oepkes D, van Kamp IL, Simon MJ, Mesman J, Overbeeke MA, Kanhai HH. Clinical value of an antibody-dependent cell-mediated cytotoxicity assay in the management of Rh D alloimmunization. Am J Obstet Gynecol 2001;184(5):1015-1020.
11. van Kamp IL, Klumper FJ, Meerman RH, Oepkes D, Scherjon SA, Kanhai HH. Treatment of fetal anemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. Acta Obstet Gynecol Scand 2004;83(8):731-737.
12. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, van Kamp IL, Oepkes D, Walther FJ, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100(3):312-316.
13. Wolf MF, Childers J, Gray KD, Chivily C, Glenn M, Jones L, Kpa M, et al. Exchange transfusion safety and outcomes in neonatal hyperbilirubinemia. J Perinatol 2020;40(10):1506-1512.
14. De Boer IP, Zeestraten EC, Lopriore E, van Kamp IL, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008;198(1):54.e1-4.
15. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr 1978;92(4):529-534.
16. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res 1992;49(1):1-6.
17. Smits-Wintjens VE, Rath ME, van Zwet EW, Oepkes D, Brand A, Walther FJ, Lopriore E. Neonatal

morbidity after exchange transfusion for red cell alloimmune hemolytic disease. Neonatology 2013;103(2):141-147.

18. Huizing K, Roislien J, Hansen T. Intravenous immune globulin reduces the need for exchange transfusions in Rhesus and AB0 incompatibility. Acta Paediatr 2008;97(10): 1362-1365.
19. Chhapola V, Sharma AG, Kanwal SK, Kumar V, Patra B. Neonatal exchange transfusions at a tertiary care centre in north India: an investigation of historical trends using change-point analysis and statistical process control. Int Health 2018;10(6):451-456.
20. Al-Lawama M, Al-Rimawi E, Al-Shibi R, Badran E. Adoption of the American Academy of Pediatrics' neonatal hyperbilirubinemia guidelines and its effect on blood exchange transfusion rate in a tertiary care center in Amman, Jordan. J Blood Med 2018;9:61-66.
21. Yu C, Li H, Zhang Q, He H, Chen X, Hua Z. Report about term infants with severe hyperbilirubinemia undergoing exchange transfusion in Southwestern China during an 11-year period, from 2001 to 2011. PLoS One 2017;12(6):e0179550.
22. Kelly AM, Williamson LM. Neonatal transfusion. Early Hum Dev 2013;89(11):855-860.
23. Zwiers C, Scheffer-Rath ME, Lopriore E, de Haas M, Liley HG. Immunoglobulin for alloimmune hemolytic disease in neonates. Cochrane Database Syst Rev 2018;3:CD003313.
24. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011;127(4):680-686.
25. Santos MC, Sa C, Gomes SC, Camacho LA, Moreira ME. The efficacy of the use of intravenous human immunoglobulin in Brazilian newborns with rhesus hemolytic disease: a randomized double-blind trial. Transfusion 2013; 53(4):777-782.
26. Stainsby D, Jones H, Wells AW, Gibson B, Cohen H, Group SS. Adverse outcomes of blood transfusion in children: analysis of UK reports to the serious hazards of transfusion scheme 1996-2005. Br J Haematol 2008;141(1):73-79.

Chapter five

Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth

Isabelle M.C. Ree
Masja de Haas
Rutger A. Middelburg
Carolien Zwiers
Dick Oepkes
Anske (J.)G. van der Bom
Enrico Lopriore

British Journal of Haematology 2019;186(4):565-573.

Abstract

Infants with haemolytic disease of the foetus and newborn (HDFN) often require erythrocyte transfusions the first three months of life. We aimed to evaluate the incidence, timing and potential predictors of transfusion-dependent anaemia. An observational cohort of 298 term and near-term infants with severe HDFN treated with or without intrauterine transfusion (IUT) was evaluated.

Transfusions were administered to 88% (169/193) of infants with IUT and 60% (63/105) without IUT. The following potential predictors were associated with less anaemia: K compared to D immunisation (odds ratio [OR] 0.13, 95%-confidence interval [95% CI]: 0.03-0.55), higher reticulocyte count at birth (per 10 parts per thousand [‰] higher, OR 0.99, 95% CI 0.97-1.00) and exchange transfusion (OR 0.11, 95% CI 0.03-0.50). Without IUT, these variables were: lower reticulocyte count at birth (per 10‰ lower, OR 1.02, 95% CI 1.00-1.03), lower maximum bilirubin after birth (per 10 µmol/L lower, OR 1.01, 95% CI 1.01-1.02) and exchange transfusion (OR 0.07, 95% CI 0.01-0.20).

In conclusion, potential predictors for anaemia in infants with severe HDFN varied between infants treated with and without IUT and are useful to select subgroups of infants at increased risk of anaemia.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is caused by the destruction of foetal and neonatal erythrocytes and possible impairment of erythropoiesis by maternal erythrocyte alloantibodies. HDFN may result in foetal and neonatal anaemia, which may persist up to three months after birth.[1,2] Prediction of this persisting anaemia after birth and of the duration of the possible transfusion dependency are central issues in the neonatal management of haemolytic disease. Anaemia in infants with HDFN is due to ongoing haemolysis, but also due to depressed erythropoiesis (hyporegenerative anaemia) and ongoing haemolysis.[3-5] While the usually early haemolytic anaemia in HDFN is one of the major focusses of neonatal care of these infants at admission, it is often overlooked how long anaemia and transfusion dependency can persist.

The vast majority of infants with severe HDFN suffer from pronounced anaemia and require erythrocyte transfusions during the first three months after birth.[6] Several factors are associated with an increased transfusion need in HDFN, including intrauterine transfusions (IUTs), treatment with exchange transfusion, severity of HDFN, and type of blood group alloimmunisation.[6-8] IUTs are thought to suppress foetal erythropoiesis and increase the need for transfusions after birth[8], whereas exchange transfusions appear to decrease the need for transfusions.[7] However, which infants are at the highest risk of developing anaemia, the time when anaemia typically presents and how long it can last before full physiologic recovery, are unclear. These questions need to be answered to design optimal follow-up procedures for these infants, finding the balance in close (laboratory) monitoring and excessive blood sampling in an anaemic population.

HDFN is rare and the care for these infants is usually scattered and shows great variability between and within countries and regions. In the Netherlands, treatment of severe HDFN is centralised in our national referral centre. We can therefore report on a considerably sized, nearly complete and to a great extent, homogenously treated population. In this study, we aimed to evaluate the incidence and potential predictors of anaemia in the first three months of life in a population of infants with severe HDFN in order to evaluate the disease trajectory in relation to transfusion management. This knowledge can be used to prevent unnecessary blood sampling and extended diagnostics in this group of infants with increased risk for HDFN-associated anaemia.

Methods

Study population

All term and near-term infants (born ≥35 weeks of gestation) with HDFN admitted to the Leiden University Medical Centre (LUMC) between January 2006 and January 2018 were eligible for the study. The LUMC is the national referral centre for severe HDFN and intrauterine treatment in the Netherlands. The guideline used in the Netherlands as part of the routine erythrocyte antibody screening programme, indicates referral to the LUMC if laboratory parameters are above determined cut-offs. This concerns antibody titres tested in maternal serum ≥2 in K immunisation and ≥16 in D or other types of alloimmunisation, or, in case of an antibody-dependent cell-mediated cytotoxicity (ADCC) assay ≥50% in case of D immunisation and ≥30% in case of other blood group antigens.[9] Subsequently, these high-risk pregnancies are monitored by serial Doppler measurements to assess the velocity of the blood flow in the middle cerebral artery (MCA). If MCA Doppler exceeds 1.5 multiples of the median velocity or if signs of hydrops are present, treatment with IUT is indicated. One or more IUTs can be administered until 34-35 weeks' gestation.[10] In this study, an antenatal diagnosis of HDFN was defined as antibody titres or ADCC test results above the aforementioned cut-offs. In case of missing values, treatment with IUT was also considered as a valid confirmation of clinically relevant HDFN.

We excluded infants born <35 weeks of gestation and infants with incomplete follow-up data regarding anaemia and transfusions after birth, which was the primary outcome measure. We also excluded infants who participated in an ongoing randomised trial on the use of erythropoietin (EPO) to prevent anaemia in HDFN and were randomised to the treatment arm. This study started in October 2017 at the LUMC (EPO-4-Rh study, NCT03104426).

The results of the current study were separately reported for infants treated with and without IUT, since an IUT may influence anaemia occurring after birth. IUT treatment is, by its indication, a sign of very severe illness, but may also alter the (patho)physiological course in these infants, which makes comparison of infants with and without IUT treatment inappropriate. Similarly for D and K alloimmunisation, a different role in the pathogenesis has been described for K alloantibodies, which are correlated to the inhibition of foetal erythropoiesis.[7] Therefore, and taking into consideration the small sample sizes of other types of alloimmunisation, extra analyses were performed excluding all types of alloimmunisation other than D.

Data collection

Data was extracted from the hospital's patient database, including medical files and laboratory outcomes. Follow-up data on transfusions after discharge from the LUMC were collected from referral hospitals after written consent was obtained from the parents or caregivers. All collected data were coded for analysis. The following maternal and neonatal data were recorded: type of alloimmunisation (D, C, c, E or K), treatment with IUT and number of IUTs, gestational age at first IUT, foetal haemoglobin level prior to first IUT, mode of delivery, gestational age at birth, birth weight, neonatal sex, neonatal haemoglobin level, bilirubin level, reticulocyte count at birth, treatment with erythrocyte transfusions and number of transfusions, neonatal age at first transfusion in days, neonatal haemoglobin level prior to first transfusion, and treatment with exchange transfusion. The study protocol and analysis plan were approved by the ethical committee of the LUMC.

Definitions and transfusion policy

As there are no uniform haemoglobin thresholds to define anaemia in newborns, anaemia was defined as a need for one or more erythrocyte transfusions in this study. The current transfusion guideline of the department, implemented in February 2014, recommends a transfusion in term infants with HDFN when haemoglobin levels fall below 10.5 g/dL (6.5 mmol/L) for day 0-6, below 8.9 g/dL (5.5 mmol/L) for day 7-13, and below 7.2 g/dL (4.5 mmol/L) from day 14 onwards. The former guideline recommended transfusion when haemoglobin levels fell below 9.6 g/dL (6.0 mmol/L) for day 7-13, and below 8.0 g/dL (5.0 mmol/L) from day 14 onwards. A transfusion of 15 ml/kg irradiated packed erythrocytes less than 5 days old was advised throughout the study period, with a haematocrit of 0.50-0.65 L/L.

Primary and secondary outcome

Primary outcome was the incidence of infants with HDFN treated for anaemia after birth i.e. those who received one or more erythrocyte transfusions. Secondary outcomes were the mean number of transfusions per infant and the mean number of days after birth before the first transfusion. Furthermore, potential predictors of anaemia were identified in subgroups of HDFN treated with and without IUT(s).

Statistical analysis

Anaemia and transfusion outcomes for infants treated with and without IUT were reported. The following variables known from literature as potential predictors of anaemia were compared

between infants with and without anaemia: sex, gestational age at birth, type of blood group alloimmunisation, treatment with an exchange transfusion, maximum bilirubin after birth, and reticulocyte level at birth. All these variables were also included in a multivariable logistic model. Separate models were run for infants with and without IUT treatment. The type of blood group alloimmunisation is divided in D immunisation, K immunisation and "other".

Results are presented as odds ratios (OR) with 95%-confidence intervals (95% CIs). An area under the curve (AUC) measure of the regression models is reported and a Kaplan-Meier curve depicting the transfusion free interval was presented. Statistical analyses were performed using IBM SPSS Statistics (version 23.0; SPSS Inc, Chicago, IL).

Results

During the studied period, 347 infants with an antenatal diagnosis of HDFN were born and admitted to the neonatal intensive care unit (NICU) of the LUMC. A total of 49 infants were excluded, of which 27 infants were excluded for a gestational age <35 weeks, and 21 infants were excluded based on missing data regarding anaemia and transfusion dependency after discharge from the LUMC. One exclusion was due to participation in the EPO-4-Rh trial and randomisation for EPO treatment. A total of 298 infants fulfilled the eligibility criteria and were included in the study (Figure 1) and follow-up was complete for 93% (277/298) of the infants. Of the 21 infants excluded based on missing data, 7 were treated with IUT and 14 were not treated with IUT, the types of alloimmunisation in this group were as follow: 13 infants were diagnosed with D immunisation, 2 with K, 4 with c, 1 with C and 1 with E.

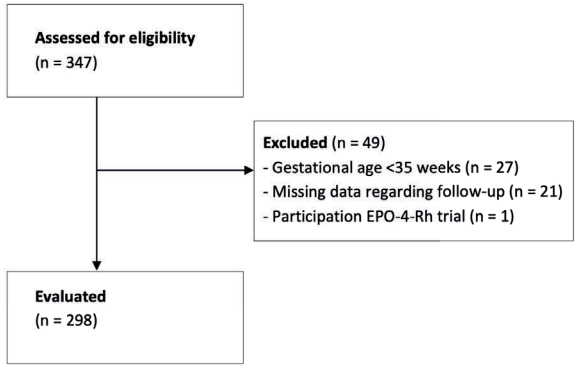


Figure 1. Flowchart of study participants

Baseline characteristics

Baseline characteristics of the cohort are presented in Table 1. The majority of the cohort was treated with IUT (193/298, 65%). Among infants who had been treated with IUT, the mean haemoglobin level at birth was 12.8 g/dL (standard deviation [SD] 2.8), the median reticulocyte count was 18 parts per thousand (‰; interquartile range [IQR] 3-56) and the median number of IUTs was 3 (IQR 2-3). Among infants not treated with IUT the mean haemoglobin level at birth was 14.2 g/dL (SD 3.4) and the median reticulocyte count was 84‰ (IQR 59-134).

Table 1. Baseline characteristics of infants with HDFN

	IUT (n = 193)	**No-IUT (n = 105)**
Male - n (%)	118 (61)	65 (62)
Caesarean delivery - n (%)	43 (22)	21 (20)
Gestational age at birth (weeks) - median (IQR)	36.0 (36.0-37.0)	37.0 (36.0-37.0)
Birth weight (grams) - mean ± SD	2902 ± 369	3000 ± 484
Haemoglobin level at birth (g/dL) - mean ± SD	12.8 ± 2.8[a]	14.2 ± 3.4
Reticulocyte count at birth (‰) - median (IQR)	18 (3-56)[b]	84 (59-134)
Number of IUTs - median (IQR)	3.0 (2.0-4.0)	-
Gestational age at first IUT (weeks) - median (IQR)	28.6 (24.2-31.8)	-
Haemoglobin level at first IUT (g/dL) - mean ± SD	7.1 ± 2.5[c]	-
D alloimmunisation - n (%)	148 (77)	76 (72)
C alloimmunisation - n (%)	0 (0)	2 (2)
c alloimmunisation - n (%)	8 (4)	13 (12)
E alloimmunisation - n (%)	1 (1)	6 (6)
K alloimmunisation - n (%)	35 (18)	4 (4)
Jka alloimmunisation - n (%)	1 (1)	2 (2)
Cw alloimmunisation - n (%)	0 (0)	2 (2)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *SD*, standard deviation.
[a] Assessed in 296/298 (99%) infants**,** 2 cases of missing value; [b] assessed in 280/298 (94%) infants, 18 cases of missing value; [c] assessed in 187/193 (97%) infants, 6 cases of missing value.

Transfusion dependency

Table 2 shows the transfusion dependency in relation to treatment with IUT(s). The incidence of anaemia (i.e. need for transfusions) after birth was 88% among infants treated with IUT and

60% among infants without IUT. The median number of transfusions was 2 (IQR 2-3) among infants with IUT and 2 (IQR 1-3) among infants without IUT. The first transfusion was given after a median of 16 days (IQR 5-27) after birth for infants with IUT, and 9 days (IQR 5-25) after birth for infants without IUT.

In case of D immunisation, IUT was performed in 66% (148/224) and 83% (187/224) received at least one transfusion after birth. In case of K immunisation, 90% (35/39) was treated with IUT and 72% (28/39) was anaemic after birth and received at least one transfusion. In the combined group of C, c and E immunisation, an IUT was performed in 29% (9/30) and 52% (16/30) of infants were treated with a transfusion after birth.

Table 2. Transfusions dependency in infants with HDFN with and without treatment with intrauterine transfusion

	IUT (n = 193)	**No-IUT (n = 105)**
Infants treated with transfusions - n (%)	169/193 (88)	63/105 (60)
Number of transfusions per infant - median (IQR)[a]	2 (2-3)	2 (1-3)
Infants received:		
1 transfusion - n (%)	40/169 (24)	27/63 (43)
2 transfusions - n (%)	62/169 (37)	16/63 (25)
3 transfusions - n (%)	39/169 (23)	12/63 (19)
4 transfusions - n (%)	19/169 (11)	5/63 (8)
5 transfusions - n (%)	5/169 (3)	3/63 (5)
6 transfusions - n (%)	4/169 (2)	0/63 (0)
Days after birth until first transfusion - median (IQR)[a,b]	16 (5-27)	9 (5-25)
Haemoglobin level at first transfusion (g/dL) - median (IQR)[a,c]	7.9 (6.8-8.9)	7.9 (7.2-8.9)
Infants treated with exchange transfusions - n (%)	31/193 (16)	16/105 (15)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number.
[a] In infants treated with transfusion(s), n=232; [b] assessed in 229/232 (99%) infants, 3 cases of missing value; [c] assessed in 223/232 (96%) infants, 9 cases of missing value.

Predictors for anaemia among infants treated with an IUT

In univariable analysis among infants treated with IUT, three variables were associated with less anaemia: K immunisation, compared with D immunisation (16 vs 33%, OR 0.35, 95% CI 0.14 to 0.92), a higher reticulocyte count at birth (10 vs 50‰, OR 0.99, 95% CI 0.98 to 1.00)

and treatment with exchange transfusion (13 vs 38%, OR 0.25, 95% CI 0.10 to 0.64). There were no statistically significant differences between anaemic and non-anaemic infants for sex, D or other types of immunisation, gestational age at birth and maximum bilirubin after birth (Table 3).

In multivariable analysis, the same three variables were associated with less anaemia: K immunisation, compared to D immunisation (OR 0.15, 95% CI 0.04 to 0.59), a higher reticulocyte count at birth (per 10‰ higher, OR 0.99, 95% CI 0.97 to 1.00) and treatment with exchange transfusion (OR 0.11, 95% CI 0.03 to 0.58). The AUC of this model is 0.83, 95% CI 0.76 to 0.91.

Predictors for anaemia among infants not treated with IUT

Among infants who had not been treated with IUT, three variables were associated with less anaemia: occurrence of the combined group of non-D, non-K types of immunisation (15 vs 38%, OR 0.24, 95% CI 0.09 to 0.63), a lower reticulocyte count at birth (100 vs 60‰, OR 1.01, 95% CI 1.00 to 1.02), and a lower maximum bilirubin after birth (290 vs 260 µmol/L, OR 1.01, 95% CI 1.00 to 1.02). The other variables, sex, D or K alloimmunisation, and treatment with exchange transfusion, were not statistically associated with anaemia after birth (Table 4).

In multivariable analysis, three variables were associated with less anaemia: a lower reticulocyte count at birth (per 10‰ lower, OR 1.02, 95% CI 1.00 to 1.03), a lower maximum bilirubin count after birth (per 10 µmol/L lower, OR 1.01, 95% CI 1.01 to 1.02), and treatment with exchange transfusion (OR 0.07, 95% CI 0.01 to 0.20). The AUC of this model is 0.83, 95% CI 0.75 to 0.91.

Table 3. Predictors for anaemia in infants with HDFN in infants treated with intrauterine transfusion

	Transfusion (n = 169)	No transfusion (n = 24)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	105/169 (62)	13/24 (54)	1.39 (0.59-3.28)	1.75 (0.63-4.82)
Type of blood group alloimmunisation				
D - n (%)	134/169 (79)	14/24 (58)		
K - n (%)	27/169 (16)	8/24 (33)	0.35 (0.14-0.92)[b]	0.13 (0.03-0.55)
Other - n (%)	8/169 (5)	2/24 (8)	0.42 (0.08-2.16)[b]	0.48 (0.08-2.91)
Gestational age at birth (per week)[a]	36.0 (36.0-37.0)	37.0 (36.0-37.0)	0.61 (0.56-1.06)	0.73 (0.38-1.39)
Reticulocyte count at birth (per 10‰)[a]	10 (0-50)	50 (10-80)	0.99 (0.98-1.00)	0.99 (0.97-1.00)
Maximum bilirubin after birth (per 10 µmol/L)[a]	220 (165-280)	235 (150-308)	1.00 (0.99-1.00)	1.00 (0.99-1.01)
Exchange transfusion - n (%)	22/169 (13)	9/24 (38)	0.25 (0.10-0.64)	0.11 (0.03-0.50)
Number of IUTs (per IUT)[a]	3 (2-4)	2 (1-3)	1.33 (0.92-1.91)	1.29 (0.78-2.13)

CI, confidence interval; *HDFN*, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (IQR) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses; [b] as compared to the risk of anaemia in D immunisation.

Table 4. Predictors for transfusions in infants with HDFN in infants not treated with intrauterine transfusion

	Transfusion (n = 63)	No transfusion (n = 42)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	40/63 (64)	25/42 (60)	1.18 (0.53-2.64)	1.56 (0.56-4.39)
Type of blood group alloimmunisation				
D - n (%)	53/63 (84)	23/42 (55)		
K - n (%)	1/63 (2)	3/42 (7)	0.15 (0.01-1.47)[b]	0.41 (0.01-26.19)
Other - n (%)	9/63 (15)	16/42 (38)	0.24 (0.09-0.63)[b]	0.31 (0.09-1.04)
Gestational age at birth (per week)[a]	37.0 (36.0-37.0)	37.0 (36.0-38.0)	0.66 (0.40-1.08)	1.05 (0.57-1.92)
Reticulocyte count at birth (per 10‰)[a]	100 (60-140)	60 (40-93)	1.01 (1.00-1.02)	1.02 (1.00-1.03)
Maximum bilirubin after birth (per 10 µmol/L)[a]	290 (260-310)	260 (178-295)	1.01 (1.00-1.02)	1.01 (1.01-1.02)
Exchange transfusion - n (%)	7/63 (11)	9/42 (21)	0.46 (0.16-1.35)	0.07 (0.01-0.20)

CI, confidence interval; *HDFN*, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (IQR) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses; [b] as compared to the risk of anaemia in D immunisation.

Transfusion-free survival

Among infants treated with IUT, 12% did not develop anaemia with a need for transfusion (Table 2). The Kaplan-Meier curve presents the transfusion-free interval (Figure 2), which showed a steep decline of the risk for a first transfusion towards 45 days after birth. In this group 97% (188/193) had a first transfusion before 45 days after birth, the median age a first transfusion was given was 16 days, as mentioned above. The highest age was 55 days. The highest age at which a transfusion was given in infants treated with IUT that received multiple transfusions, was exactly 100 days after birth, with a median of 49 days (IQR 44-54). The median transfusion interval was therefore from 16 to 49 days after birth.

Among infants not treated with IUT, 40% did not develop anaemia with a need for transfusion (Table 2). Again the Kaplan-Meier curve showed a decline of the risk for a first transfusion towards 45 days after birth. In this group, 99% (104/105) had a first transfusion before 45 days after birth, the median age at which a first transfusion was given was 9 days. The highest age was 61 days. The highest age at which a transfusion was given in infants not treated with IUT that received multiple transfusions, was 83 days after birth, with a median of 30 days (IQR 26-34). The median transfusion interval was therefore from 9 to 30 days after birth.

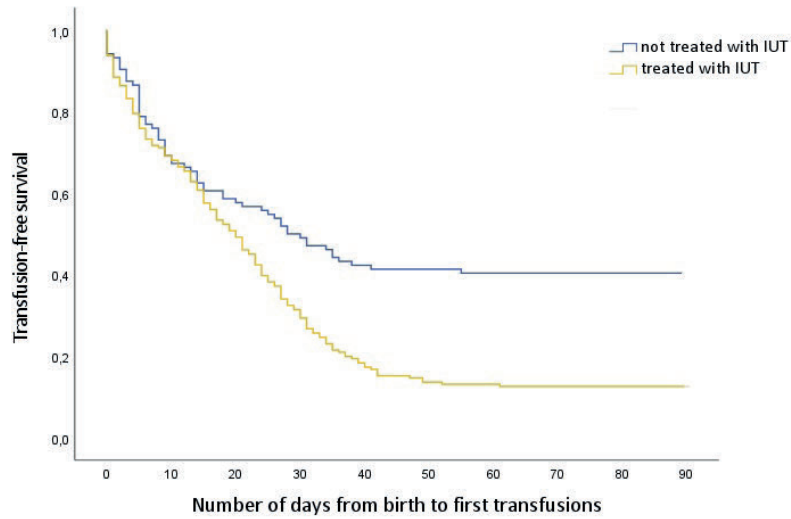


Figure 2. Kaplan-Meier curve, transfusion-free interval
IUT; intrauterine transfusion.

D-immunisation

As most infants had HDFN due to anti-D immunisation (75% of the study population), additional analyses were done after exclusion of other types of immunisation to verify the robustness of our findings in a more homogeneous population (Tables 5-8). In univariable analysis, a higher reticulocyte count at birth (10 vs 80 ‰, OR 0.97, 95% CI 0.96 to 0.99), maximum bilirubin after birth (245 vs 285 µmol/L, OR 0.99, 95% CI 0.99 to 1.00), and treatment with exchange transfusion (16 vs 57%, OR 0.14, 95% CI 0.04 to 0.44) were statistically significantly associated with less anaemia in the subgroup of D immunised pregnancies treated with IUT(s), Table 7. In multivariable analysis, the reticulocyte count at birth remained statistically associated with anaemia and transfusion dependency (OR 0.98, 95% CI 0.96 to 1.00). Treatment with exchange transfusion was not statistically significant in this subpopulation on multivariable analysis.

Among infants not treated with IUT, univariable analysis showed no variables that were statistically significant associated with anaemia in the D-immunised subgroup. Multivariable analysis showed an association between a lower reticulocyte count at birth (per 10‰ lower, OR 1.01, 95% CI 1.00 to 1.02), lower maximum bilirubin after birth (per 10 µmol/L lower, OR 1.01, 95% CI 1.00 to 1.02), and exchange transfusion (OR 0.12, 95% CI 0.02 to 0.58), Table 8.

Table 5. Baseline characteristics of infants with HDFN due to D immunisation

	IUT (n = 148)	**No-IUT (n = 76)**
Male - n (%)	91 (62)	48 (63)
Caesarean delivery - n (%)	34 (23)	18 (24)
Gestational age at birth (weeks) - median (IQR)	36.0 (36.0-37.0)	37.0 (36.0-37.0)
Birth weight (grams) - mean ± SD	2859 ± 367	3075 ± 479
Haemoglobin level at birth (g/dL) - mean ± SD	12.7 ± 2.8[a]	13.7 ± 3.4
Reticulocyte count at birth (‰) - median (IQR)	22 (3-58)[b]	90 (65-134)
Number of IUTs - median (IQR)	2.0 (2.0-4.0)	-
Gestational age at first IUT (weeks) - median (IQR)	29.1 (24.6-32.1)	-
Haemoglobin level at first IUT (g/dL) - mean ± SD	7.2 ± 2.5[c]	-

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *SD*, standard deviation.
[a] Assessed in 222/224 (99%) infants, 2 cases of missing value; [b] assessed in 214/224 (96%) infants, 10 cases of missing value; [c] assessed in 143/148 (97%) infants, 5 cases of missing value.

Table 6. Transfusion dependency in infants with HDFN due to D immunisation with and without treatment with intrauterine transfusion

	IUT (n = 148)	**No-IUT (n = 76)**
Infants treated with transfusions - n (%)	134/148 (91)	53/76 (70)
Number of transfusions per infant - median (IQR)[a]	2 (2-3)	2 (1-3)
Infants received:		
1 transfusion - n (%)	23/134 (17)	23/53 (43)
2 transfusions - n (%)	50/134 (37)	13/53 (25)
3 transfusions - n (%)	35/134 (24)	10/53 (19)
4 transfusions - n (%)	17/134 (11)	5/53 (9)
5 transfusions - n (%)	5/134 (3)	2/53 (4)
6 transfusions - n (%)	4/134 (3)	0/53 (0)
Days after birth until first transfusion - median (IQR)[a,b]	16 (4-27)	9 (4-21)
Haemoglobin level at first transfusion (g/dL) - median (IQR)[a,c]	7.9 (6.9-9.0)	7.8 (7.1-8.8)
Infants treated with exchange transfusions - n (%)	29/148 (20)	14/76 (18)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number.
[a] In infants treated with transfusion(s) after birth, n=187; [b] assessed in 184/187 (98%) infants, 3 cases of missing value; [c] assessed in 179/187 (96%) infants, 8 cases of missing value.

Table 7. Predictors for anaemia in infants with HDFN due to D immunisation in infants treated with intrauterine transfusion

	Transfusion (n = 134)	No transfusion (n = 14)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	8/134 (62)	8/14 (57)	1.22 (0.40-3.72)	1.63 (0.39-6.77)
Gestational age at birth (per week)[a]	36.0 (36.0-37.0)	37.0 (36.0-37.0)	0.46 (0.21-0.99)	0.45 (0.19-1.01)
Reticulocyte count at birth (per 10‰)[a,]	10 (0-50)	80 (48-100)	0.97 (0.96-0.99)	0.98 (0.96-1.00)
Maximum bilirubin after birth (per 10 µmol/L)[a]	245 (180-290)	285 (230-343)	0.99 (0.99-1.00)	1.00 (0.99-1.01)
Exchange transfusion - n (%)	21/134 (16)	8/14 (57)	0.14 (0.04-0.44)	0.26 (0.05-1.43)
Number of IUTs (per IUT)[a]	2 (2-4)	2 (1-2)	1.74 (1.00-3.00)	1.48 (0.72-3.05)

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (*IQR*) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses.

AUC = 0.90 (95% CI 0.81 to 0.98)

Table 8. Predictors for anaemia in infants with HDFN due to D immunisation in infants not treated with intrauterine transfusion

	Transfusion (n = 53)	No transfusion (n = 23)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	35/53 (66)	13/23 (57)	1.50 (0.55-4.07)	2.01 (0.66-6.14)
Gestational age at birth (per week)[a]	37.0 (36.0-37.0)	37.0 (36.0-38.0)	0.89 (0.50-1.58)	1.06 (0.57-1.95)
Reticulocyte count at birth (per 10‰)[a]	90 (65-130)	80 (60-110)	1.01 (1.00-1.02)	1.01 (1.00-1.02)
Maximum bilirubin after birth (per 10 µmol/L)[a]	280 (260-310)	270 (230-340)	1.00 (1.00-1.01)	1.01 (1.00-1.02)
Exchange transfusion - n (%)	7/53 (13)	7/23 (30)	0.35 (0.11-1.15)	0.12 (0.02-0.58)

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (*IQR*) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses

AUC = 0.77 (95% CI 0.66 to 0.88)

Discussion

In this study we quantified the need for erythrocyte transfusions in infants suffering from severe HDFN in relation to treatment with and without IUT(s) and identified potential predictors for anaemia and ongoing transfusion dependency. The overall occurrence of anaemia after birth was 78%. We differentiated between infants treated with and without IUT and analysed the time-dependent pattern of anaemia and transfusion in these infants. Among infants treated with IUT, the incidence of anaemia was 88% and the first transfusion was administered 16 days after birth. Among infants not treated with IUT, the incidence of anaemia was 60% and the first transfusion was administered 9 days after birth. In both groups, transfusion management only incidentally started at a later time point than 45 days.

As part of the pathophysiology of HDFN, it has been described that the ongoing destruction of foetal erythrocytes is compensated by extramedullary haematopoiesis and an erythroblastosis in the infant's blood. Indeed, in the infants that did not receive IUT(s), a *high* reticulocyte count at birth was strongly associated with anaemia. Also, a higher maximum bilirubin count after birth was significantly associated with anaemia. We hypothesise that in infants not treated with IUT(s), a high reticulocyte count at birth reflects an active compensatory mechanism for ongoing haemolysis (supported by higher maximum bilirubin counts after birth) and thus indicates more severe haemolytic illness in this group of non-treated infants than those infants with a low reticulocyte count at birth, resulting in a high transfusion dependency. Among infants treated with IUT, we found that the reticulocyte counts at birth showed an inverse association. After IUT, a *low* reticulocyte count at birth was strongly associated with anaemia. Treatment with IUT(s), by definition, indicates a more severe foetal haemolytic course of HDFN, possibly explaining the more severe neonatal disease trajectory with anaemia and a long period of transfusion dependency. IUTs have been described to suppress erythropoiesis[8,11] and we indeed found a *low* reticulocyte count at birth after IUT treatment. Previous studies also showed a correlation between a lower reticulocyte count at birth and anaemia.[8,12,13] However, low reticulocyte counts are seen in both K and D alloimmunisation, while K alloimmunisation shows a less severe course of anaemia after birth despite a higher IUT need.[7] The possible suppressive effect of IUTs can therefore not fully explain the observed anaemia and long transfusion dependency. A potential explanation could be that in K alloimmunisation even low titres are associated with severe impairment of the foetal erythropoiesis[14] and these infants may therefore show a more rapid decline in antibodies after birth and shorter period of transfusion dependency, despite treatment with IUT(s). D alloimmunisation is associated with high antibody titres, which could explain the prolonged suppressive effects of IUT

treatment, especially in absence of treatment with exchange transfusion that could reduce the high antibody load. Unfortunately, the anti-RBC antibody titres were not routinely sampled in the infants after birth, preventing further speculations. In future studies, prospective measurements of anti-RBC antibody titres could yield additional crucial information.

In multivariable analysis in both the IUT-treated infants and in the infants without IUT, a consistent statistically significant association was found between treatment with exchange transfusion and anaemia. The rate of exchange transfusion among infants treated with and without IUT (19% and 16%) was similar to findings of other recent studies conducted after implementation of the guideline by the American Academy of Pediatrics (AAP) in 2004.[15-18] Exchange transfusions are indicated to remove excessive bilirubin from the neonatal circulation if intensive phototherapy is insufficient. Exchange transfusions have additional effects as it are also erythrocyte transfusions in itself and remove maternal antibodies and IgG coated erythrocytes from the neonatal circulation. Removal of antibodies may theoretically reduce the ongoing process of haemolysis and therefore reduce the risk of anaemia.

The Kaplan-Meier analysis to determine the transfusion-free interval is of high clinical relevance, as it illustrates how the risk of a first transfusion after birth decreased over time and stabilised after approximately 45 days for the vast majority of the infants treated with IUT (97%) and those not receiving IUT (99%). The current expert opinion in the Netherlands is to closely follow-up on haemoglobin and reticulocyte counts on a weekly basis up to three months of life. For this study population, this means that weekly blood sampling test for haemoglobin measurements were performed in 22% of infants that never developed anaemia. Our data suggest that a follow-up period of approximately 45 days is sufficient if, by that time, no transfusions have been necessary. We recommend a shortened follow-up period for infants in good clinical condition, with proper instruction to caregivers.

Overall the study has several strengths and limitations. One of the major strengths is the Dutch setting which enabled us to establish a large, homogenous and near complete collection of data and follow-up records of an increasingly rare disease. However, the results must be carefully interpreted in the context of our population, as it is a selection of (very) severe HDFN cases as result of the referral guidelines in the Netherlands. Infants not treated with IUT, particularly the less severe cases that did not require foetal therapy, were probably more likely to have been admitted elsewhere. It may also be difficult to translate these results to other populations, as there is a great international variability in haemoglobin transfusion thresholds.[19] Other limitations of this study are the small numbers of alloimmunisation other

than D. Additionally, this study did not take other causes of anaemia into consideration, such as infection or blood loss due to obstetric causes or bleeding in the neonatal period,[20] although these were rare in this population. The effect of the most frequent cause of anaemia in infants, prematurity, was eliminated in the study by assessing a (near) term cohort.

In conclusion, severe anaemia is a common complication of HDFN and clinicians need to be aware of this risk and actively monitor the process of haemolysis and recuperation of erythropoiesis in HDFN. IUT treatment, by its definition and indications, reflects a severe haemolytic process in the foetus and, after birth, these infants are at high risk for anaemia. In particular, low reticulocyte counts at birth in infants treated with IUT were highly correlated with anaemia. In contrast, in infants without IUT treatment, high reticulocyte counts at birth, were strongly associated with anaemia and transfusion dependency which may indicate severe haemolysis despite no antenatal need of treatment.

If infants develop transfusion dependent anaemia after birth, the disease trajectory can extent to the first three months of life. However, to prevent unnecessarily long periods of follow-up and frequent blood sampling tests for haemoglobin levels, we showed with our large cohort of severe cases that follow-up can be closed at 45 days if there are no signs of anaemia and need for transfusion at that time for infants in good clinical condition with proper instruction to caregivers.

References

1. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Hemolytic disease of the fetus and newborn. Vox Sang 2015;109(2):99-113.
2. Urbaniak SJ, Greiss MA. RhD hemolytic disease of the fetus and the newborn. Blood Rev 2000;14(1):44-61.
3. al-Alaiyan S, al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med 1999;27:112-115.
4. Strand C, Polesky HF. Delayed anemia in erythroblastosis fetalis. Minn Med 1972;55:439-441.
5. Widness JA, Lowe LS, Stevenson DK, Vreman HJ, Weinier CP, Hayde M, Pollak A. Direct relationship of foetal carboxyhaemoglobin with haemolysis in alloimmunised pregnancies. Pediatr Res 1994;35(6):713-719.
6. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, Oepkes D, Walther FJ, Lopriore E. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010;99:65-70.
7. Rath ME, Smits-Wintjens VEHJ, Lindenburg ITM, Brand A, van Kamp IL, Oepkes D, Walther FJ, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100:312-316.
8. De Boer IP, Zeestraten EC, Lopriore E, van Kamp IL, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008;198:54e1-4.
9. Dutch Society for Obstetrics and Gynaecology (NVOG): Erythrocyte immunisation and pregnancy. available from https://www.nvog.nl/wp-content/uploads/2018/03/Erytrocytenimmunisatie-en-zwangerschap_.pdf. 2009.
10. Zwiers C, van Kamp I, Oepkes D, Lopriore E. Intrauterine transfusion and non-invasive treatment options for haemolytic disease of the foetus and newborn - review on current management and outcome. Expert Rev Haematol 2017;10:337-344.
11. Egberts J, van Kamp IL, Kanhai HH, Meerman RH, Giordano PC, Gravenhorst JB. The disappearance of fetal and donor red blood cells in alloimmunised pregnancies: a reappraisal. Br J Obstet Gynaecol 1997;104(7):818-824.
12. Goodrum LA, Saade GR, Belfort MA, Carpenter RJ Jr, Moise KJ Jr. The effect of intrauterine transfusion on fetal bilirubin in red cell alloimmunization. Obstet Gynecol 1997;89(1):57-60.
13. Millard DD, Gidding SS, Socol ML, MacGregor SN, Dooley SL, Ney JA, Stockman JA. Effects of intravascular, intrauterine transfusion on prenatal and postnatal hemolysis and erythropoiesis in severe fetal isoimmunization. J Pediatr 1990;117(3):447-454.
14. Slootweg YM, Lindenburg ITM, Koelewijn JM, van Kamp IL, Oepkes D, de Haas M. Predicting anti-Kell-mediated hemolytic disease of the fetus and newborn: diagnostic accuracy of laboratory management Am J Obstet Gynecol 201;219(4):393.e1-e8.
15. American Academy of Pediatrics Subcommittee on H. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316.
16. McGlone L, Simpson JH, Scott-Lang C, Cameron AD, Brennand J. Short-term outcomes following intrauterine transfusion in Scotland. Arch Dis Child Fetal Neonatal Ed 2011;96(1):F69-70.
17. Pasman SA, Claes L, Lewi L, Van Schoubroeck D, Debeer A, Emonds M, Geuten E, De Catte L, Devlieger R. Intrauterine transfusion for fetal anemia due to red blood cell alloimmunization: 14 years experience in Leuven. Facts Views Vis Obgyn 2015;7(2):129-136.

18. Smits-Wintjens VEHJ, Walther FJ, Rath ME, Lindenburg ITM, te Pas AB, Kramer CM, Oepkes D, et al. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011;127:680-686.

19. Howarth C, Banerjee J, Aladangady N. Red Blood Cell Transfusion in Preterm Infants: Current Evidence and Controversies. Neonatology 2018;114(1):7-16.

20. Colombatti R, Sainati L, Trevisanuto D. Anemia and transfusion in the neonate. Semin Fetal Neonatal Med 2016;21(1):2-9.

Chapter six

Randomised controlled trial on the use of EPO to reduce postnatal transfusions in neonates with red blood cell alloimmunisation treated with intrauterine transfusions (protocol)

Isabelle M.C. Ree
Masja de Haas
Nan van Geloven
Dick Oepkes
Vivianne E.H.J. Wintjens
Anske (J.)G. van der Bom
Enrico Lopriore

In preparation

Abstract

Background

Up to 80% of infants with haemolytic disease of the foetus and newborn (HDFN) treated with intrauterine transfusion(s), require at least one postnatal transfusion for anaemia during the first 3 months of life. Erythropoietin (EPO) deficiency is considered a possible contributing factor to postnatal hyporegenerative anaemia in HDFN, and exogenous EPO administration may lower the postnatal transfusion-dependency in this population.

Objective

To evaluate the effect of darbepoetin alfa on the need for postnatal red blood cell transfusions in neonates with HDFN due to red cell alloimmunisation treated with IUT.

Study design

A total of 44 (near)-term infants admitted to the Leiden University Medical Centre (LUMC) with HDFN and treated with IUT will be included in this randomised controlled trial. Patients will be randomised to treatment with EPO (darbepoetin alfa, or Aranesp®) subcutaneously at a dosage of 10 µg/kg once a week for a period of 8 weeks (intervention), or "standard care". The primary outcome is the number of postnatal red blood cell transfusions required per infant after birth.

Discussion

The postnatal burden for infants affected by severe HDFN and their parents and caregivers is still high, with intensive follow-up after birth and often readmittance to hospital for one or multiple postnatal transfusions. This study evaluates the potential role for EPO-treatment in the postnatal management of HDFN.

Trial registration

Working title "EPO-4-Rh"-study, identifier NCT03104426, available at https://clinicaltrials.gov/ct2/show/NCT03104426.

Background

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which maternal alloantibodies lead to the destruction of foetal erythrocytes. The mainstay of antenatal treatment of foetal anaemia HDFN is (serial) intrauterine transfusion (IUT).[1,2] The mainstay of postnatal treatment in HDFN is (1) intensive phototherapy and, if necessary, exchange transfusion to treat hyperbilirubinaemia and prevent kernicterus, and (2) postnatal transfusions to treat anaemia.[3]

Up to 88% of children with HDFN treated with IUT require at least one postnatal transfusion for anaemia during the first 3 months of life, with a median of two postnatal transfusions involving two hospital admissions per infant. [4-6] Postnatal anaemia can be divided in early (up to 7 days of life) anaemia due to ongoing haemolysis and late anaemia (7 days - 3 months of age). Late anaemia in neonates with HDFN may be due to depressed erythropoiesis (hyporegenerative anaemia) and/or persisting (intra-marrow) destruction of erythrocytes by remaining antibodies.[4,7,8] Hyporegenerative anaemia occurs in particular in neonates treated with several IUTs.[4,7,9] Other contributing factors for late anaemia 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).[3] Finally, erythropoietin (EPO) deficiency is also considered as a possible contributing factor to late anaemia.[10-15]

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.[14,16-18] 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.[10-15] However, other authors found that EPO may be less effective than expected.[19] Due to the lack of evidence, routine use of EPO is currently not recommended.[3] To determine a role for administration of EPO in this group of patients, a randomised controlled clinical trial of sufficient sample size is required to evaluate the effect of exogenous EPO on the prevention of postnatal anaemia in HDFN, as potential alternative for red blood cell transfusions.[15] 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.

Methods

Objective

The primary objective of this study is to investigate whether EPO is effective in reducing the incidence of late anaemia in children with HDFN treated with IUT, and therefore in decreasing the number of postnatal transfusions per child, compared to a control group of children receiving standard care without darbepoetin alfa (or Aranesp®, a long acting agent of EPO) treatment.

Study population

All (near) term children (gestational age ≥ 35 weeks) with HDFN (due to D, C, c, E, K or other red blood cell alloimmunisation) treated with IUT and admitted to the Leiden University Medical Centre (LUMC) after October 2017 are eligible for the study. The LUMC is the single national referral centre in the Netherlands for pregnancies complicated by maternal red blood cell alloimmunisation. A prenatal national screening program in the Netherlands indicates referral to the LUMC in case of elevated antibody titres tested in maternal serum ≥1/2 in K immunisation and ≥1/16 in D or other types of alloimmunisation or in case of an elevated antibody-dependent cell-mediated cytotoxicity (ADCC) assay ≥ 50% in case of D immunisation and ≥ 30% in case of other blood group antigens. These pregnancies are monitored by serial Doppler measurements to assess the velocity of the middle cerebral artery (MCA), which is considered the most accurate non-invasive predictor of foetal anaemia. If MCA Doppler exceeds 1.5 multiples of the median, or if signs of hydrops are present, treatment with IUT is indicated. Intrauterine transfusion is usually continued until 34-35 weeks' gestation to progress these pregnancies to term.[20] In general, labour and admission of the child to the Neonatology department of the LUMC is highly recommended in these pregnancies. Approximately 15 eligible patients are treated in the LUMC annually.

Transfusion policy

Postnatal anaemia in this study is defined as a need for one or more erythrocyte transfusions. The LUMC transfusion guideline recommends a postnatal transfusion of 15 ml/kg irradiated erythrocytes in full-term neonates with HDFN when haemoglobin levels fall below 10.5 g/dL (6.5 mmol/L, day 0-6), below 8.9 g/dL (5.5 mmol/L, day 7-13) and below 7.2 g/dL (4.5 mmol/L, from day 14). These cut-off values are communicated with referral hospitals after discharge from the LUMC and the research team is in contact with these hospitals to ensure transfusions are given according to these cut-off values.

Study design

The EPO-4-Rh study is a single centre randomised controlled trial. The trial is registered with ClinicalTrials.gov, identifier NCT03104426. Included children are randomised at birth to treatment with darbepoetin alfa (intervention group) or "standard care", with 1:1 allocation, to be randomised in varying blocks of 4 and 6, no stratification is applied. In the treatment group, darbepoetin alfa is administered subcutaneously at a dosage of 10 μg/kg once a week, starting at approximately day 7, for a period of 8 weeks. Treatment is administered during weekly home visits in the treatment arm after discharge from the LUMC. No concomitant therapy with folate (0.25 mg/day) is given (standard practice), concomitant iron therapy is given if ferritin level drops below 75 microg/l (standard practice). Weekly routine measurements of complete blood counts (including haemoglobin level, haematocrit and reticulocyte count) will be performed in both groups (standard practice). EPO is discontinued if haemoglobin level is $\geq$ 13 g/dL after at least 4 weeks of treatment with EPO. In EPO-treated children, blood pressure will be measured for safety reasons at onset of treatment, after four weeks and eight weeks. Monthly measurements of liver enzymes (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transferase (γGT) and lactate dehydrogenase (LDH)) will also be performed in all groups (standard practice). In addition, the neonatal EPO-level will be determined at birth in both groups. The number of postnatal transfusions received during the first 3 months of life and haemoglobin levels prior to the postnatal transfusion are recorded. After initial discharge from the LUMC, postnatal red blood cell transfusions are recommended to be performed when haemoglobin levels fall below aforementioned cut-off values of haemoglobin. Because of these clearly defined guideline and haemoglobin measures, blinding of caregivers to treatment allocation and use of a placebo was not deemed necessary.

Data collection

Collected data, including history of pregnancy, neonatal clinical records from the initial hospitalisation at the LUMC, and records from additional admissions and outpatient clinic visits in other hospitals than the LUMC, are collected for each included patient with written consent of parents or caregivers. Data are upon collection blinded and entered into an online secured database (CASTOR) by a member of the research team with in this database blinding of treatment allocation. The data manager and statistician involved are blinded as well. After all data is collected and entered in the database, and after data cleaning, the database will be locked and the data will be unblinded to all parties involved.

End points

The primary end point is the number of postnatal red blood cell transfusions per child from birth up to 3 months of life. Secondary end points are: the percentage of children requiring a postnatal transfusion up to 3 months of life; time from birth to first postnatal transfusion (days); haemoglobin level at first postnatal transfusion (g/dL); number of days of hospitalisation and readmission(s) associated with erythrocyte transfusion(s); course of haemoglobin up to 3 months of life.

Sample size calculation

Based on the (scarce) results in the literature, we expect a 50% reduction in the median number of total postnatal red blood cell transfusions per patient with EPO treatment, from a median of 2 to 1. For sample size calculation we hypothesised a shift in the distribution of number of transfusions per child as depicted in Figure 1. The distribution in the 'care as usual' group are based on data from 2000-2014. Based on these expected frequencies, group sample sizes of 21 infants achieve 81% power to detect a difference of 1.1 between the null hypothesis that both group means are 1.9 and the alternative hypothesis that the mean of the EPO group is 0.8 with estimated group standard deviations of 1.5 and 0.9 and with a significance level (alpha) of 0.05 using a two-sided Mann-Whitney test. The drop-out percentage is estimated at 5%, adding (42/0.95 = 44) 1 child to each group's sample size.

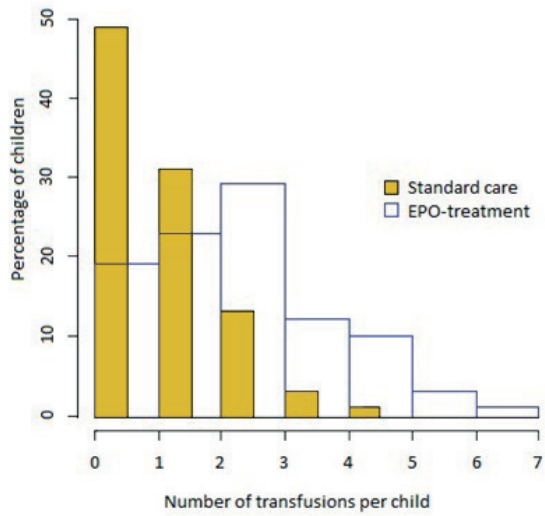


Figure 1. Hypothesised distribution of number of postnatal transfusions

Discussion

The postnatal burden for children affected by severe HDFN and their parents and caregivers is still high, with intensive follow-up after birth and often readmittance to hospital for one or multiple postnatal transfusions. Of children treated with IUT, 88% receives one or more red blood cell transfusions, with a median of two transfusions per child, although some children require up to six transfusions after birth.[6] The number of postnatal transfusions does not show a decline over time with improvement of neonatal care.[5,6] This randomised controlled study evaluates the potential role for EPO (darbepoetin alfa) in the postnatal course of HDFN, and will determine whether EPO is an effective agent to reduce postnatal transfusion dependency and medicalisation of these patients.

References

1. Van Kamp IL, Klumper FJ, Meerman RH, Oepkes D, Scherjon SA, Kanhai HH. Treatment of fetal anemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. Acta Obstet Gynecol Scand 2004;83:731-737.
2. Weiner CP, Williamson RA, Wenstrom KD, Sipes SL, Widness JA, Grant SS, Estle L. Management of fetal hemolytic disease by cordocentesis. II. Outcome of treatment. Am J Obstet Gynecol 1991;165:1302-1307.
3. Smits-Wintjens VE, Walther FJ, Lopriore E. Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. Semin Fetal Neonatal Med 2008;13:265-271.
4. De Boer IP, Zeestraten EC, Lopriore E, van Kamp IL, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008;198:54.
5. Rath ME, Smits-Wintjens VEHJ, Lindenburg I, Brand A, Oepkes D, Walther FJ, Lopriore E. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010;99:65-70.
6. Ree IMC, de Haas M, Middelburg RA, Zwiers C, Oepkes D, van der Bom JG, Lopriore E. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth. Br J Haematol 2019;186(4):565-573.
7. Thorp JA, O'Connor T, Callenbach J, Cohen GR, Yeast JD, Albin J, Plapp F. Hyporegenerative anemia associated with intrauterine transfusion in rhesus hemolytic disease. Am J Obstet Gynecol 1991;165:79-81.
8. Koenig JM, Ashton RD, De Vore GR, Christensen RD. Late hyporegenerative anemia in Rh hemolytic disease. J Pediatr 1989;115:315-318.
9. Ree IMC, Lopriore E, Zwiers C, Böhringer S, Janssen MWM, Oepkes D, De Haas M. Suppression of compensatory erythropoiesis in hemolytic disease of the fetus and newborn due to intrauterine transfusions. Am J Obstet Gynecol 2020;223(1):119.e1-10.
10. Scaradavou A, Inglis S, Peterson P, Dunne J, Chervenak F, Bussel J. Suppression of erythropoiesis by intrauterine transfusions in hemolytic disease of the newborn: use of erythropoietin to treat the late anemia. J Pediatr 1993;123:279-284.
11. Ovali F, Samanci N, Dagoglu T. Management of late anemia in Rhesus hemolytic disease: use of recombinant human erythropoietin (a pilot study). Pediatr Res 1996;39:831-834.
12. Zuppa AA, Maragliano G, Scapillati ME, Florio MG, Girlando P, Noia G, De Santis M, et al. Recombinant erythropoietin in the prevention of late anaemia in intrauterine transfused neonates with Rh-haemolytic disease. Fetal Diagn Ther 1999;14:270-274.
13. Nicaise C, Gire C, Casha P, d'Ercole C, Chau C, Palix C. Erythropoietin as treatment for late hyporegenerative anemia in neonates with Rh hemolytic disease after in utero exchange transfusion. Fetal Diagn Ther 2002;17:22-24.
14. Ohls RK, Wirkus PE, Christensen RD. Recombinant erythropoietin as treatment for the late hyporegenerative anemia of Rh hemolytic disease. Pediatrics 1992;90:678-680.
15. Zuppa AA, Alighieri G, Calabrese V, Visintini F, Cota F, Carducci C, Antichi E, et al. Recombinant human erythropoietin in the prevention of late anemia in intrauterine transfused neonates with Rh-isoimmunization. J Pediatr Hematol Oncol 2010;32:e95-101.

16. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2006;3:CD004863.

17. Aher S, Ohlsson A. Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2006;3:CD004868.

18. Ohls RK, Kamath-Rayne BD, Christensen RD, Wiedmeier SE, Rosenberg A, Fuller J, Lacy CB, et al. Cognitive outcomes of preterm infants randomized to darbepoetin, erythropoietin, or placebo. Pediatrics 2014;133(6):1023-1030.

19. Dallacasa P, Ancora G, Miniero R, Gavella B, Brondelli L, Conte R, Salvioli GP. Erythropoietin course in newborns with Rh hemolytic disease transfused and not transfused in utero. Pediatr Res 1996;40:357-360.

20. Zwiers C, van Kamp I, Oepkes D, Lopriore E. Intrauterine transfusion and non-invasive treatment options for hemolytic disease of the fetus and newborn - review on current management and outcome. Expert Rev Hematol 2017;10(4):337-344.

Chapter seven

Necrotising enterocolitis in haemolytic disease of the newborn: a retrospective cohort study

Isabelle M.C. Ree*
Anne M. de Grauw*
Vincent Bekker
Masja de Haas
Arjan B. te Pas
Dick Oepkes
Annemieke (J.)M. Middeldorp
Enrico Lopriore

*Authors contributed equally

Vox Sanguinis 2020;115(2):196-201.

Abstract

Background and objectives

Necrotising enterocolitis (NEC) is a common and often severe gastrointestinal emergency in newborn infants. While usually affecting (very) premature infants, an association between NEC and haemolytic disease of the foetus and newborn (HDFN) has been suggested. HDFN may be an additional risk factor to develop NEC. The objective of this study was to evaluate the occurrence of NEC in infants affected with moderate to severe HDFN in a large single centre cohort as compared to a broad population of infants without HDFN.

Materials and methods

Retrospective cohort study of medical records of neonates with and without HDFN, with a gestational age at birth ≥30 weeks and ≤38 weeks, and admitted to the Leiden University Medical Centre between January 2000 and December 2016.

Results

A total of 3284 patient records of infants born in the study period were reviewed and 317 cases of HDFN were identified. The incidence of NEC was significantly higher among infants with HDFN compared to infants without HDFN: 4/317 affected infants (1.3%) vs 11/2967 affected infants (0.4%, relative risk 3.40, 95% confidence interval 1.09-10.63).

Conclusions

We observed a higher incidence of NEC in an overall late preterm to near term population of infants with moderate to severe HDFN, compared to infants born without HDFN. The clinician taking care of an HDFN affected infant should be cautious of this higher risk.

Introduction

Necrotising enterocolitis (NEC) is one of the most common gastrointestinal emergencies in newborn infants and is defined by ischaemic necrosis of the intestine with a high mortality rate.[1] Epidemiologic studies have identified multiple risk factors for the development of NEC, such as prematurity and low birth weight.[2,3] NEC in late prematurity is rare, but could develop as a complication among otherwise predisposed infants.[4] 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.[5] Haemolytic disease of the foetus and newborn (HDFN) can influence peripheral oxygenation and may also influence the gastrointestinal system because of high bilirubin levels. Potential associations between NEC and HDFN or treatment for HDFN have been reported.[6,7]

Severe HDFN is a nowadays rare condition caused by an incompatibility between maternal and foetal red blood cell antigens. Maternal alloantibody formation against foetal red blood can cause foetal anaemia and, if left untreated, foetal hydrops and death.[8] Antenatal treatment in HDFN is mainly based on intrauterine erythrocyte transfusion (IUT) to the foetus in case of severe foetal anaemia, and in rare cases, early intravenous immunoglobulin (IVIg) treatment is started in pregnancy.[9] After birth, HDFN is characterised by hyperbilirubinaemia and ongoing anaemia, which can last up to three months of age.[10] Postnatal treatment of HDFN can include phototherapy, exchange transfusions, erythrocyte transfusions, and in some centres, IVIg is administered.

In the population of HDFN affected infants, several of the aforementioned predisposing factors for NEC are present. In the literature, both antenatal and postnatal treatments of HDFN have been associated with the occurrence of NEC.[11-13] Due to the rarity of severe HDFN and of NEC in late premature and near term infants, it is very difficult to conclusively investigate causative associations between HDFN, the treatment of HDFN, and the occurrence of NEC. It is therefore critical to report cases of NEC and HDFN to unravel the underlying pathophysiology of HDFN and NEC and their shared characteristics. The objective of this study was to evaluate and report the occurrence of NEC and various clinical characteristics in a large population of infants affected by HDFN, as compared to a broad population of infants admitted to the neonatal intensive care unit (NICU) of our centre without HDFN.

Methods

We conducted a retrospective cohort study of medical records of neonates with and without HDFN. The medical records of all live-born infants with a gestational age at birth ≥30 weeks and ≤38 weeks admitted to our NICU between January 2000 and December 2016 were reviewed for this study. Outborn patients and infants with major congenital abnormalities were excluded.

In the Netherlands, all pregnant women are routinely screened for the presence of alloantibodies in pregnancy and maternal blood samples with a positive screening result are sent to one of the two national referral laboratories (Sanquin Diagnostic Services or the Special Institute for Blood group Investigations (BIBO)). Thereafter, the clinical relevance of the antibody is evaluated by assessing the antibody specificity, and by assessing whether the foetus is antigen-positive. If the foetus is positive, the risk on foetal haemolysis is assessed by serially determining the antibody titre and antibody-dependent cell-mediated cytotoxicity (ADCC). The cut-off values for moderate to severe HDFN and referral to a specialised centre are set at an ADCC assay ≥50% in case of D immunisation and ≥30% in case of other blood group antigens, or antibody titres tested in maternal serum ≥1:16 in D and ≥1:2 in K immunisation.[14] These high-risk pregnancies are referred to the Leiden University Medical Centre (LUMC), the national referral centre for HDFN and intrauterine treatment in the Netherlands. At the LUMC, these pregnancies are monitored by serial Doppler measurements to assess the velocity of the blood flow in the middle cerebral artery (MCA). If MCA Doppler exceeds 1.5 multiples of the median (MoM) velocity or if signs of hydrops are present, treatment with IUT is indicated. Planned delivery at the LUMC and neonatal admission to the NICU of the LUMC is recommended for all high-risk pregnancies.

The primary outcome was the relative risk to develop NEC in case of (moderate to severe) HDFN compared to a population of infants without HDFN delivered at a similar gestational age range. The characteristics of infants with HDFN and NEC were specified in more detail for full comprehension of these cases. *P*-values or other indicators of statistical significance were not reported as the various variables are not defined as outcome measures in this study, but have a descriptive nature.

NEC was defined according to Bell's criteria ≥ stage 2A and diagnosis was confirmed radiographically in the presence of pneumatosis intestinalis.[15] Several clinical characteristics that were derived from literature as potential predisposing factors for NEC were collected of this population. The following perinatal and neonatal data were collected: gender, gestational

age at birth, birth weight, small for gestational age (SGA) (defined as birth weight <10th centile for gestational age), multiple/singleton, mode of delivery, perinatal asphyxia, presence of umbilical or central venous catheter, NEC (≥2A according to Bell's criteria),[16] hypotension (treated with inotropics), proven sepsis (confirmed by a positive blood culture), need for ventilation, need for erythrocyte transfusion, need for exchange transfusion and mortality. In case of HDFN, the following additional data were collected: type of alloimmunisation, treatment with IUT (number of IUTs) and antenatal or postnatal treatment with IVIg.

The data were analysed using IBM SPSS Statistics (version 25.0; SPSS Inc, Chicago, IL). The primary outcome was reported as a relative risk with a 95% confidence interval. Other data are reported as n (%), mean or median (interquartile range [IQR] / standard deviation [SD]), depending on the underlying distribution of the data.

All data were collected as part of standard practice and were retrieved from the medical status from infants and mothers with written consent from parents and caregivers for infants with HDFN. For all (other) infants admitted to the NICU, parents and caregivers are informed of the use of medical data for retrospective research and can choose to opt out. The ethical committee of the LUMC reviewed the research proposal and confirmed the observational nature of the study and provided a waiver for further ethical consent.

Results

After excluding patients with major congenital abnormalities, the data of 3284 infants was included in the study. Of this population, 317 (9.7%) infants were admitted for HDFN and were compared to infants without HDFN (n=2967).

Table 1 shows the baseline characteristics of the cohort, infants admitted with HDFN were more often male (61.5%) and had a median gestational age at birth of 36 weeks (IQR 36-37). Infants without HDFN had a median gestational age of 34 weeks (IQR 31-36 weeks). Infants with HDFN had, in accordance with the higher median gestational age at birth, a higher median birth weight of 2900 grams (IQR 2610-3203) than unaffected infants, which had a median birth weight of 2222 grams (IQR 1640-2715). Infants with HDFN had also lesser occurrence of being born SGA (2.5 vs 12%) and were more often singletons (97.4 vs 39.7%) compared to infants without HDFN.

Table 1. Baseline characteristics

	HDFN (n = 317)	No HDFN (n = 2967)
Male - n (%)	195 (61.5)	1543 (52.0)
Caesarean delivery - n (%)	83 (26.2)	1268 (42.7)
Gestational age at birth (weeks) - median (IQR)	36 (36-37)	34 (31-36)
Birth weight (grams) - median (IQR)	2900 (2610-3203)	2222 (1640-2715)
SGA - n (%)	8 (2.5)	356 (12.0)
Singleton - n (%)	309 (97.4)	1178 (39.7)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *n*, number; *SGA*, small for gestational age.

During the study period, there were 4 (1.3%) infants with NEC in the HFDN population and 11 infants (0.4%) with NEC in the population without HDFN of similar gestational age (Table 2). The relative risk of developing NEC is therefore 3.40 (1.3/0.4; 95% confidence interval 1.09-10.63). Clinical characteristics with a potential association with NEC varied among HDFN-affected and HDFN-unaffected infants and are shown in Table 2. Umbilical catheters, erythrocyte transfusions and exchange transfusions were more present among infants affected by HDFN.

Table 2. Clinical outcomes

	HDFN (n = 317)	No HDFN (n = 2967)
NEC - n (%)	4 (1.3)	11 (0.4)
Need for ventilation - n (%)	6 (1.9)	260 (8.8)
Hypotension requiring inotropics - n (%)	3 (0.9)	66 (2.2)
Umbilical catheter - n (%)	103 (32.5)	482 (16.2)
Central venous catheter - n (%)	14 (4.4)	187 (6.3)
Erythrocyte transfusion - n (%)	76 (24.0)	155 (5.2)
Exchange transfusion	52 (16.4)	2 (0.1)
Proven sepsis - n (%)	14 (4.4)	140 (4.7)
Perinatal asphyxia - n (%)	1 (0.3)	34 (1.1)
Mortality - n (%)	2 (0.6)	15 (0.5)

HDFN, haemolytic disease of the foetus and newborn; *n*, number; *NEC*, necrotising enterocolitis.

When comparing infants with NEC and HDFN to infants with NEC without HDFN, NEC occurred at a higher median gestational age at birth and higher birth weight among HDFN-affected

infants compared to infants that suffered from NEC without HDFN (34 vs 31 weeks and 1879 vs 1201 grams; respectively, Table 3). A significant portion of infants with NEC without HDFN was born SGA compared to infants with NEC and HDFN (45.5 vs 0.0%). Clinical features between affected infants with and without HDFN are shown in Table 3.

Table 3. Characteristics of NEC cases

	NEC HDFN (n = 4)	**NEC no HDFN (n = 11)**
Male - n (%)	1 (25.0)	4 (36.3)
Caesarean delivery - n (%)	3 (75.0)	8 (72.7)
Gestational age at birth (weeks) - median (IQR)	34 (31-35)	31 (30-33)
Birth weight (grams) - median (IQR)	1879 (1289-2052)	1201 (807-1606)
SGA - n (%)	0 (0.0)	5 (45.5)
Need for mechanical ventilation - n (%)	1 (25.0)	4 (36.3)
Hypotension - n (%)	0 (0.0)	2 (18.1)
Umbilical catheter - n (%)	2 (50.0)	7 (63.4)
Central venous catheter - n (%)	3 (75.0)	7 (63.4)
Erythrocyte transfusion - n (%)	2 (50.0)	3 (27.3)
Exchange transfusion - n (%)	1 (25.0)	0 (0.0)
Proven sepsis - n (%)	0 (0.0)	3 (27.5)
Perinatal asphyxia - n (%)	0 (0.0)	0 (0.0)
Mortality - n (%)	1 (25.0)	2 (18.1)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *n*, number; *NEC*, necrotising enterocolitis*; SGA*, small for gestational age.

Of the infants with HDFN, four infants developed NEC, which are specified in Table 4. In the entire HDFN population of this study, the majority was diagnosed with D immunisation (75.4%) and treated with IUT (64.4%). A total of 52 patients (16.4%) needed exchange transfusion. Postnatal erythrocyte transfusions were given to 76 (24.0%) patients during the initial admission to the LUMC after birth. Compared to the overall HFDN population, the infants that developed NEC had a lower gestational age (range 30-35 weeks, compared to a median of 36 weeks) and lower birth weight (range 1147-2055 gram, compared to a median of 2900 gram). Of the four cases, there were two cases of D alloimmunisation, one of K immunisation and one of c immunisation. All affected infants were treated with IUT, varying between a total number of 1-5 IUTs. One infant was treated with antenatal IVIg treatment, one received

exchange transfusion after birth (before the onset of NEC) and two infants received erythrocyte transfusions after birth (after the onset of NEC). Three infants were treated conservatively and survived, one required surgery for NEC and did not survive.

Table 4. NEC in HDFN population

	CASE NO. 1	**CASE NO. 2**	**CASE NO. 3**	**CASE NO. 4**	**HDFN population** (n=317)
Gender	Male	Female	Female	Female	Male 195 (61.5)[a]
Gestational age at birth (weeks)	33	34	30	35	36 (36-37)[b]
Birth weight (grams)	2043	1715	1147	2055	2900 (2610-3203)[b]
Type of alloimmunisation	D	D	c	K	239 (75.4) D, 41 (12.9) K, 37 (11.7) other
Treated with IUT	Yes	Yes	Yes	Yes	204 (64.4)[a]
Number of IUTs	5	2	1	5	2 (0-3)[b]
Antenatal IVIg treatment	No	No	No	Yes	9 (2.8)[a]
Postnatal IVIg treatment	No	No	No	No	41 (12.9)[a]
Haemoglobin level at birth (g/dL)	9.0	9.5	19.7	13.9	13.1 ± 3.4[c]
Erythrocyte transfusion	Yes	Yes	No	No	76 (24.0)[a]
Exchange transfusion	Yes	No	No	No	52 (16.4)[a]
Onset of NEC (day of life)	4	3	10	5	-
Treatment of NEC	Conservative	Conservative	Conservative	Surgical	-
Survival	Yes	Yes	Yes	No	315 (99.4)[a]

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *IVIg*, intravenous immunoglobulin; *NEC*, necrotising enterocolitis.
[a] Number (%); [b] median (interquartile range); [c] mean ± standard deviation.

Discussion

The objective of this study was to evaluate and compare the occurrence of NEC in infants affected by HDFN to infants admitted to the NICU of our centre without HDFN. During the 17-year study period, we found a relative risk to develop NEC for infants with HDFN of 3.40 (95%-confidence interval 1.09-10.63), although the absolute risk of developing NEC in our HDFN cohort was low (1.3%). These findings are consistent with previous reports suggesting HDFN as an additional predisposing factor for NEC.[7]

We report on a cohort of moderate to severe HDFN, as defined by antibody titres and ADCC values, as mild HDFN is no indication for referral to the NICU of the LUMC. Cases of mild HDFN can only be expected at our NICU as a secondary (or tertiary) diagnosis, and in the data of infants without HDFN may therefore be under reported. However, mild HDFN with a low ADCC and/or antibody titre and no need for intrauterine monitoring or transfusions, or intensive phototherapy, is unlikely to bias the potential relation with NEC.

We acknowledge that the infants admitted to the NICU without HDFN are a reference group with a broad range of pathology and treatments, but despite the great heterogeneity in the population without HDFN, a higher occurrence of NEC was found in HDFN affected infants. Despite the lower occurrence of NEC among infants without HDFN, known risk factors for NEC were actually more present, including a lower median gestational age at birth, lower median birth weight and these infants were more often SGA at birth.

Furthermore, while we report on a very large sample of HDFN, we acknowledge the low incidence of NEC in this study population and are cautious to draw conclusions with merely 15 infants with NEC in our study distributed over a long time period. This low occurrence rate of NEC in our study prevents reliable risk factor analysis and perhaps the study should, in terms of accuracy, be considered as an extended cases series. However, for hypothesis-generating purposes, the data were carefully further explored. One can speculate that the higher risk to develop NEC might be due to the relative hypoxia as part of the pathophysiology of HDFN itself, but NEC has also been suggested to be a complication of IUT or (postnatal) IVIg treatment.[6] These suggested associations are complicated as, for example, IUT and IVIg treatment may have a treatment-related effect on the foetal circulation and oxidative state, but are in itself also signs of more severe HDFN and thus anaemia, which is also associated with NEC.[17] The high occurrence of erythrocyte (exchange) transfusions in the infants with HDFN could similarly be seen as potential predisposing factor for NEC, but is also a reflection of the severity of HDFN. More severely affected HDFN infants are in higher need of phototherapy and exchange transfusions (hence the higher need for umbilical catheters) and erythrocyte transfusions.[10] In our population of HDFN-affected infants, all four infants that developed NEC received IUTs and only one was treated with antenatal IVIg. Two of the four affected infants received a strikingly high number of five IUTs, while only one infant required an exchange transfusion. While the infants in this study with HDFN were actually born at a median higher gestational age and higher median birth weight compared to the infants without HDFN, the four infants that developed NEC had lower gestational ages and lower birth weights compared to the other HDFN infants. This might carefully imply that the effect of prematurity and low birth

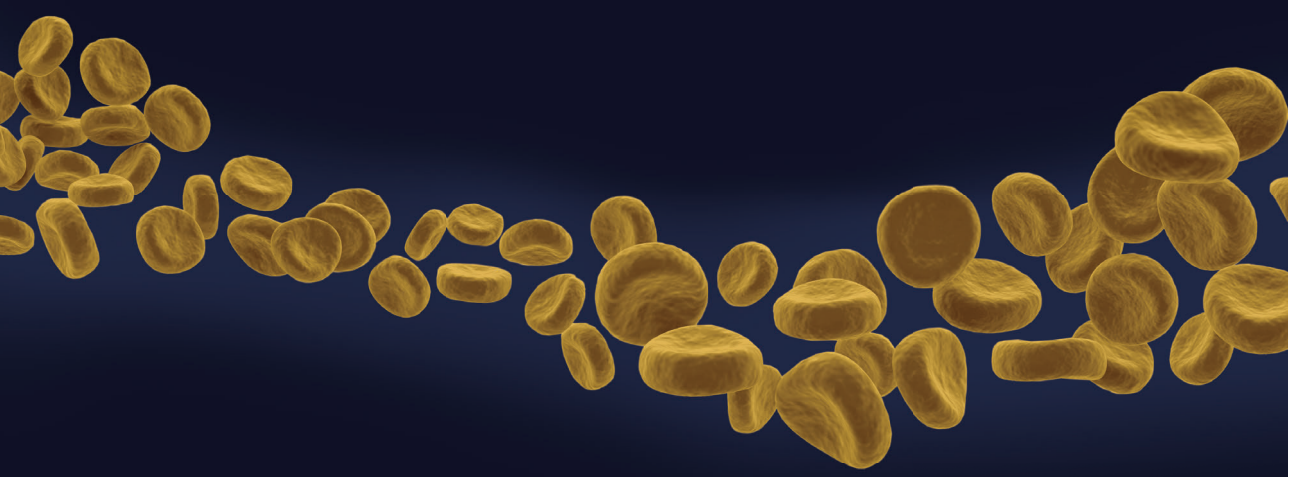
weight to develop NEC is enhanced in the presence of one or more additional predisposing factor, such as (treatment for) HDFN.

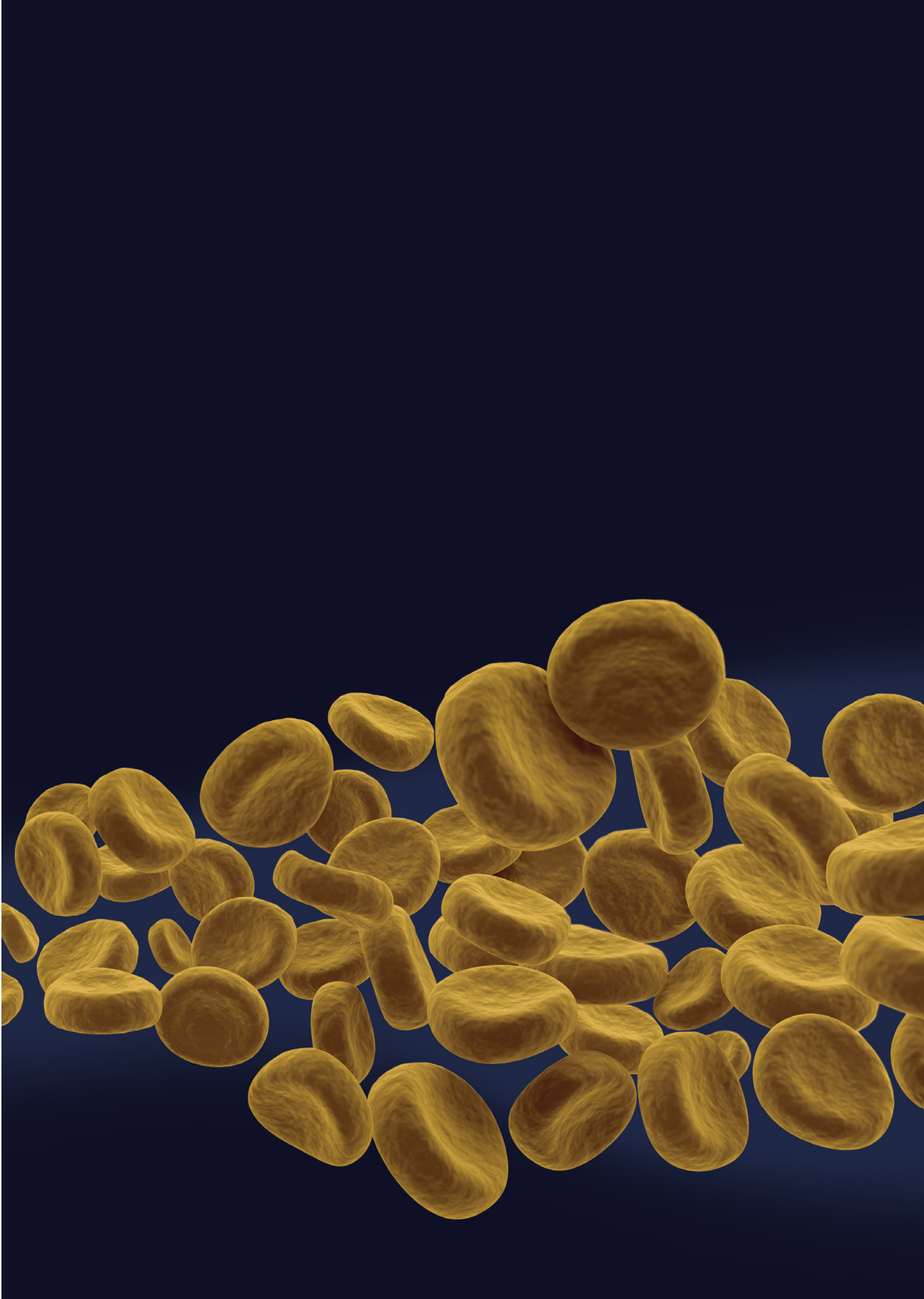
HDFN is a rare disease and due to better and more preventive measures and treatment options, the number of affected patients is small and still declining, which complicates extended research within this population. However, our centre is the national referral centre for HDFN in the Netherlands and as a result a substantial database of HDFN patients has been created, in which we try to observe and investigate rare outcomes. Our 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. The clinician taking care of an HDFN-affected infant should be cautious of this rare but serious complication and perhaps especially in otherwise predisposed infants by late prematurity or a low birth weight.

References

1. Dominguez KM and Moss RL. Necrotising enterocolitis. Clin Perinatol 2012;39(2):387-401.
2. Fitzgibbons SC, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, Lillehei C, et al. Mortality of necrotising enterocolitis expressed by birth weight categories. J Pediatr Surg 2009;44(6):1072-1075.
3. Heida FH, Stolwijk L, Loos MHJ, van den Ende SJ, Onland W, van den Dungen FAM, Kooi EMW, et al. Increased incidence of necrotising enterocolitis in the Netherlands after implementation of the new Dutch guideline for active treatment in extremely preterm infants: Results from three academic referral centers. J Pediatr Surg 2017;52(2):273-276.
4. Lambert DK, Christensen RD, Henry E, Besner GE, Baer VL, Wiedmeier SE, Stoddard RA, et al. Necrotising enterocolitis in term neonates: data from a multihospital health-care system. J Perinatol 2007;27(7):437-443.
5. Lin PW and Stoll BJ. Necrotising enterocolitis. Lancet 2006;368(9543):1271-1283.
6. Rao SC, Patole SK, Dickinson JE, Reid KP, Doherty DA. Neonatal necrotising enterocolitis following intrauterine transfusions: is there an association? J Matern Fetal Neonatal Med 2004;16(1):51-54.
7. Roig JC, Burchfield DJ. Term neonates with haemolytic disease of the newborn and necrotising enterocolitis: a report of two cases. J Perinatol 1994;14(3):201-203.
8. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the foetus and newborn. Vox Sang 2015;109(2):99-113.
9. Zwiers C, van der Bom JG, van Kamp IL, van Geloven N, Lopriore E, Smoleniec J, Devlieger R, et al. Postponing Early intrauterine Transfusion with Intravenous immunoglobulin Treatment; the PETIT study on severe haemolytic disease of the foetus and newborn. Am J Obstet Gynecol 2018;219(3):291.e1-9.
10. Ree IMC, de Haas M, Middelburg RA, Zwiers C, Oepkes D, van der Bom JG, Lopriore E. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth. Br J Haematol 2019;186(4):565-573.
11. Figueras-Aloy J, Rodríguez-Miguélez JM, Iriondo-Sanz M, Salvia-Roiges M, Botet-Mussons F, Carbonell-Estrany X. Intravenous immunoglobulin and necrotising enterocolitis ain newborns with haemolytic disease. Pediatrics 2010;125(1):139-144.
12. Teiserskas J, Bartasiene R and Tameliene R. Associations between Red Blood Cell Transfusions and Necrotising Enterocolitis in Very Low Birth Weight Infants: Ten-Year Data of a Tertiary Neonatal Unit. Medicina (Kaunas) 2019;55(1).
13. Berman L and Moss RL. Necrotising enterocolitis: an update. Semin Fetal Neonatal Med 2011;16(3):145-150.
14. Vandenbussche FP, Klumper FJ. Dutch guideline on erytrocyte immunisation and pregnancy. Dutch society for Obstetrics and Gynacology (NVOG). 2011, version 2.1.
15. Gregory KE, Deforge CE, Natale KM, Philips M, Van Marter LD. Necrotising enterocolitis in the premature infant: neonatal nursing assessment, disease pathogenesis, and clinical presentation. Adv Neonatal Care 2011;11(3):155-164.
16. Bell MJ. Neonatal necrotising enterocolitis. N Engl J Med 1978;298(5):281-282.
17. Maheshwari A, Patel RM, Christensen RD. Anaemia, red blood cell transfusions, and necrotising enterocolitis. Semin Pediatr Surg 2018;27(1):47-51.

Part 4 - Long-term outcome





Chapter eight

School performance and behavioural functioning in children after intrauterine transfusions for haemolytic disease of the foetus and newborn

Isabelle M.C. Ree
Renske M. van 't Oever
Lisette Jansen
Enrico Lopriore
Masja de Haas
Jeanine M.M. van Klink

Early Human Development 2021;157:105381.

Abstract

Aim

To investigate the school performance and behavioural difficulties in children with haemolytic disease of the foetus and newborn (HDFN) treated with intrauterine transfusion (IUT) compared to Dutch norm data.

Study design

Cross-sectional cohort study.

Subjects

Children who received one or multiple IUTs for severe Rh- or K (Kell)-mediated HDFN between January 2008 and January 2015 at the Leiden University Medical Centre in the Netherlands.

Outcome measures

School performance reports were assessed as well as behavioural difficulties as assessed with the Dutch child behavioural checklist (CBCL) by parents and caregivers and the Teacher report form (TRF) completed by teachers.

Results

A response rate of 56% (70 children, aged 5-12 years) was obtained. Grade repetition occurred in 13 cases (19%), 16 children (23%) received some form of additional help, most often support by a speech therapist (n=8), but also support for dyslexia (n=4), physical therapy (n=2) and social-emotional support (n=2). None of the children in our study group attended special-needs education. School performance levels for reading comprehension, spelling and mathematics according to the Dutch National Pupil Monitoring System were similar for the study population and Dutch norm data. 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.

Conclusion

This study shows favourable and reassuring school development in children treated with IUT in an experienced foetal-therapy centre. A normal distribution in school and behavioural development is to be expected for children with HDFN treated with IUTs.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which maternal erythrocyte alloantibodies lead to the destruction of incompatible foetal erythroid cells. This haemolytic process can lead to severe anaemia, foetal hydrops and ultimately intrauterine demise. The mainstay of antenatal treatment in HDFN is intrauterine transfusion (IUT) to correct foetal anaemia. After birth, infants affected by HDFN are prone to severe hyperbilirubinaemia and prolonged anaemia and are treated with intensive phototherapy, exchange transfusion in case of treatment failure of phototherapy, and red blood cell transfusions.[1,2]

HDFN is nowadays a rare condition due to, in particular, the introduction of RhD immunoprophylaxis and treatment with IUT. After introduction of RhD immunoprophylaxis in 1968 and its administration during pregnancy, the RhD immunisation rate dropped from 16% to 0.3%.[3] Successful IUT was first described in 1963 and has developed into a safe procedure with only 0.6% procedure-related complications and high survival rates (97%) when performed regularly in an experienced centre.[4]

The focus in HDFN switched from mere survival to long-term effects on development and impairment after severe HDFN and intrauterine treatment. As described, children affected by HDFN are exposed to severe foetal anaemia and hyperbilirubinaemia after birth, making them hypothetically prone to foetal hypoxic injury to the developing brain and varying degrees of bilirubin neurotoxicity.

A few studies with small patient numbers have reported over the years on long-term neurodevelopmental outcome after IUT, showing overall a favourable outcome. In general, these studies focussed on survival rate and gross motor function and most often included more severely affected foetuses and cases of foetal hydrops.[5-11] No follow-up studies were conducted in the last 15 years, apart from an earlier large cohort study in our centre, aimed to determine the incidence and risk factors for adverse neurodevelopmental outcome after IUT treatment between 1988 and 2008. It was found that the incidence of neurodevelopmental impairment (NDI) was 4.8% (14/291).[12]

Since this study, no long-term follow-up of these children has been performed by our (and other) centres despite continuous advancements in foetal and neonatal care of infants affected by HDFN and near elimination of severe hydrops. While severe NDI was then comparable to Dutch norm data, it is unclear if less outspoken behavioural, learning and physical difficulties occur in this group and how this potentially translates to a school environment and academic development.

The main purpose of this study is to investigate the current state of school performance and behavioural difficulties of this population by questionnaires and school results. This study will provide clinicians and parents with updated insights in long-term development of children with HDFN that were treated with IUTs, specified to school performances in comparison with Dutch norm data.

Methods

Study population

This is a cross-sectional cohort study, including all children who received one or multiple IUT for severe Rh- or K-mediated HDFN between January 2008 and January 2015 at the Leiden University Medical Centre (LUMC). Children born before 2008 were studied before.[12] The LUMC is the national referral centre for foetal therapy of severe HDFN in the Netherlands. Exclusion criteria were severe congenital anomalies unrelated to intrauterine anaemia or treatment. Due to the non-invasive nature of this study, a waiver of consent was granted by the medical ethics committee of our centre.

Procedures

The study interventions are standardised questionnaires for parents or caregivers ('child behavioural checklist', CBCL) and teachers ('teachers report form', TRF). Initial contact with parents or caregivers was made by phone, after which invitation letters to participate in the study were sent by mail. If parents or caregivers consented, they received the questionnaires by mail and were asked to give the TRF and an accompanying letter requiring school performance scores to their child's teacher. After two and four weeks, parents or caregivers were reminded to respond, once by phone and once by mail. If we were unable to make initial contact with parents or caregivers by phone, an invitation letter was sent by mail and contact information was checked with the child's general practitioner registered in the medical file.

Further relevant demographic and perinatal data was collected for descriptive purposes from the medical files of the children in a computerised database with permission of parents or caregivers. Data included are: type of alloimmunisation, gestational age at IUT, number of IUTs, gestational age at birth, gender, birth weight, bilirubin levels at birth and neonatal outcome including hydrops at birth and number of exchange transfusions because of severe hyperbilirubinaemia.

The main study parameter was the overall school performance score of children treated before birth with IUT(s) for HDFN compared to Dutch norm scores. Secondary endpoints were the prevalence of behavioural difficulties as assessed with the CBCL and TRF.

Questionnaires

The CBCL and TRF are questionnaires assessing behavioural competency and behavioural problems in children within the past six months.

The CBCL for ages 6-18 and TRF for ages 6-18 consist of 120 items describing the behaviour of school-aged children. Parents and teachers answered the items similar to the three-point Likert scale on the CBCL for ages 1½-5. Based on their answers, eight syndrome scales were calculated: (1) anxious/depressed behaviour, (2) withdrawn/depressed behaviour, (3) somatic complaints, (4) social problems, (5) thought problems, (6) attention problems, (7) rule-breaking behaviour and (8) aggressive behaviour.

All syndrome scales together form a total problem scale, which can be divided into the subscale internalising problem behaviour (anxious/depressed behaviour, withdrawn/depressed behaviour and somatic complaints) and the subscale externalising problem behaviour (rule-breaking behaviour and aggressive behaviour).[13,14]

Standardized t-scores were obtained, where higher scores indicate higher levels of problem behaviour. For the syndrome scale, t-scores <65 were considered normal, t-scores between 65-70 borderline clinical and t-scores >70 clinical behavioural problems. For the total problem, internalising and externalising scale, t-scores <60 were considered normal, t-scores between 60-75 borderline clinical and t-scores >65 clinical behavioural problems. Our study population was compared to Dutch norm data, the norm clinical score is 10% in these scales.[13,14]

School performance scores

Part of the CBCL and TRF include questions about the attendance of regular education, special-needs education and grade repetition. The results of the study population were compared to Dutch norm data as obtained from the Dutch Central Bureau for Statistics (CBS) on enrolment in special-needs primary education (CBS 2019-2020) and the grade repeat rate (CBS 2019-2020).[15,16] Data on school performance as reported in the Dutch National Pupil Monitoring System was additionally obtained for reading comprehension (the understanding and interpretation of written language), spelling and arithmetic/mathematics. This system consists of tests taken at specific moments during the school year. The results can be translated into

ability scores to compare an individual child to their age matched peers. The ability scores are divided into 5 levels, A to E with A being the top 25% highest scoring children and E being the 10% lowest performing children.[17]

Demographic data

In addition to the questionnaires, parents were asked to provide demographic data on their highest level of education and current form of employment. The level of education was classified in 'low', 'intermediate' or 'high'. Low education was defined as primary and secondary school education, intermediate education as intermediate vocational school education and high education as higher vocational school and university education. In 2019, 40% of the Dutch population aged 25-64 years had completed either higher vocational school or university.[18]

Statistics

Descriptive results were presented as number of cases and percentages or as mean ± standard deviation or median (interquartile range) depending on the distribution of the data. Binomial tests for proportions were conducted to compare the percentage of children in special-needs education, the grade repetition rate and the percentages of children in each level of school performance to their Dutch peers. All statistical analyses were performed using IBM SPSS Statistics (version 26.0; SPSS Inc, Chicago, IL).

Results

A total of 125 live-born children were treated with IUTs in the study period and were approached to participate in this study. There were no children with severe congenital anomalies. Written consent was obtained from the parents or caregivers of 70 children (56%), the parents or caregivers of 8 children did not consent in participating (6%). Contact was established with parents or caregivers of 16 children (13%), but they never returned the questionnaires. No contact could be made with parents or caregivers, or the primary care physician of 31 children (25%), these cases were classified as loss to follow up.

When written consent was obtained, a complete response with both the CBCL form as the TRF form and school results was received for 57 children. Incomplete responses occurred in 13 cases. The study population is presented in a flowchart, Figure 1.

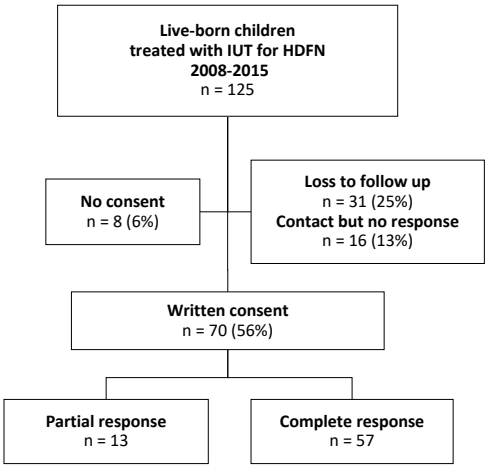


Figure 1. Flowchart of study population
HDFN, haemolytic disease of the foetus and newborn; *n*, number.

Perinatal outcome

The baseline perinatal characteristics of the children included (n=70) and lost to follow up (n=31) are presented in Table 1. Of the included children, the majority was male (64%) and the median gestational age at birth was 36 weeks (IQR 36-37 weeks). The median number of IUTs per child was 2 (IQR 2-4 IUTs), one child presented with hydrops at birth. In more than half of the cases (66%) RhD was the causative type of alloimmunisation. A quarter of children was treated with one or more exchange transfusions (26%). 45% of mothers reported educational levels classified as 'high' and 48% of fathers.

Table 1. Baseline characteristics

	Analysed group (n = 70)	**Loss to follow up (n = 31)**
Type of alloimmunisation[a]		
D alloimmunisation - n (%)	46 (66)	27 (87)
K alloimmunisation - n (%)	19 (27)	4 (13)
c/C alloimmunisation - n (%)	4 (6)	0 (0)
E alloimmunisation - n (%)	1 (1)	0 (0)
Gestational age at first IUT - weeks[a]	30 (25-32)	26 (23-32)
Number of IUT(s) per foetus[b]	2 (2-4)	3 (2-4)
Haemoglobin at first IUT - g/dL[b]	5 (3-6)	7 (5-8)
Hydrops present at birth - n (%)	1 (1)	0 (0)
Gestational age at birth - weeks[b]	36 (36-37)	36 (35-37)
Birth weight - grams[b]	2999 (2720-3265)	2710 (2508-2940)
Male gender - n (%)	45 (64)	20 (65)
Neonates treated with ET - n (%)	18 (26)	6 (19)
Maternal education - n (%)[c]		-
Low	6 (10)	
Intermediate	29 (45)	
High	29 (45)	
Paternal education - n (%)[c]		
Low	5 (8)	
Intermediate	28 (44)	
High	31 (48)	-

ET, exchange transfusion; *IUT*, intrauterine transfusion; *n*, number.
[a] Rounds up to more than 100% due to rounding; [b] data presented as median (interquartile range); [c] 6 missing values due to incomplete response.

Grade progression

In this study population, grade repetition occurred in 13 cases (19%). Dutch primary school has eight grades, ranging from 1 (4-year olds) to 8 (12-year olds). Groups 1 and 2 are similar to kindergarten, where from group 3 on, children learn to read, write and learn arithmetic/ mathematics. Three children repeated grade 1, three repeated group 2, five children repeated group 3, one child repeated group 4 and one child repeated group 6.

This is significantly higher than the Dutch norm of 10.3%[16] (*p*=.020). 16 children (23%) received some form of additional help, most often support by a speech therapist (n=8), but also support for dyslexia (n=4), physical therapy (n=2) and social-emotional support (n=2). None of the children in our study group attended special-needs primary education, as compared to 2.6% of the Dutch norm population[15] (*p*=.176).

Academic performance

The different school performance levels for reading comprehension, spelling and mathematics according to the Dutch National Pupil Monitoring System were compared with the study population (Table 2). The scores of the study population are distributed similarly from level A (highest) to E (lowest) as the Dutch norm data for reading comprehension and spelling. Compared to the Dutch norm data, the study population more often scored in the range of level A in mathematics (44% vs 25%, *p*=.002). A *p*-value $<$.003 was considered significant after Bonferroni correction.

Table 2. Academic performance

	Study population	Dutch norm population	*p*-value
Reading comprehension (n = 49)[a]			
A	12 (25)	25%	.544
B	13 (27)	25%	.456
C	10 (20)	25%	.288
D	9 (18)	15%	.310
E	5 (10)	10%	.550
Spelling (n = 53)			
A	17 (32)	25%	.151
B	9 (17)	25%	.114
C	11 (21)	25%	.296
D	11 (21)	15%	.162
E	5 (9)	10%	.561
Mathematics (n = 55)			
A	24 (44)	25%	**.**002
B	14 (26)	25%	.521
C	8 (15)	25%	.045
D	4 (7)	15%	.070
E	5 (9)	10%	.524

[a] Numbers presented as n (%).
A *p*-value <.003 was considered significant after Bonferroni correction

Behavioural outcome

A total of 63 (90%) parents that gave written consent for participation filled out the CBCL forms and 56 (80%) teachers completed the TRF forms. Teachers and parents reported a diagnosis of dyslexia for four children, there was no specific mentioning of attention deficit hyperactivity disorder (ADHD). When asked directly whether the student (for teachers) or child (for parents) has a physical or mental disability, all teachers and parents answered with 'no'.

There were no significant differences between the scores of the CBCL forms and the Dutch norm data. From the TRF forms, the clinical score of Externalising problems 1 (2%) and Total problem scale 0 (0%) were both significantly lower than the Dutch norm (*p*-value of .020 and .003, respectively). The separate scores per syndrome scale are presented in Table 3.

Table 3. Behavioural outcome; CBCL and TRF scores 6-18 years

	Study population	Dutch norm population	*p*-value
CBCL (n = 63)			
Internalising problems, clinical score - n (%)	8 (13%)	10%	.293
Syndrome scales T-scores (IQR)			
Anxious/Depressed	50 (50-57)		
Withdrawn/Depressed	52 (50-60)		
Somatic complaints	53 (50-52)		
Externalising problems, clinical score - n (%)	4 (6)	10%	.232
Syndrome scales T-scores (IQR)			
Rule-breaking behaviour	50 (50-52)		
Aggressive behaviour	50 (50-52)		
Total problems, clinical score - n (%)	3 (5)	10%	.113
TRF (n = 56)			
Internalising problems, clinical score - n (%)	8 (14)	10%	.193
Externalising problems, clinical score - n (%)	1 (2)	10%	.020
Total problems, clinical score - n (%)	0 (0)	10%	.003

CBCL, child behavioural checklist; *n*, number; *TRF*, teachers report form.

Discussion

In this study we aimed to assess long-term outcomes of HDFN treated with IUTs, specified to school performance scores as compared to Dutch norm data. We found that no children in the study population attend special-needs education. Twice as many children in the study group repeated a grade compared to Dutch normative data, although it must be noted that 6 of the 13 children that repeated a grade, did so already at the young age of 4 or 5 years. A quarter of children received 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. In mathematics, the study population performed even better compared to 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. We found no trend in remarkable behaviour or problems as reported by parents and teachers.

When conducting this study, we hypothesised that children born with severe HDFN show more difficulty in their educational development. The severe foetal anaemia that these children

were exposed to, may have caused foetal hypoxic injury to the developing brain similar to the negative effect of hypoxia during birth. The latter is known to have a negative effect on verbal and cognitive performance.[19] In addition, these children were exposed to often severe hyperbilirubinaemia after birth, and may have experienced varying degrees of bilirubin neurotoxicity. Nonetheless, the children of this study population have scores similar to age-related peers.

The response rate in this study was 56%, which is not uncommon for questionnaire studies.[20] Several attempts were made to contact parents or caregivers, including the verification of contact information with the child's registered general practitioner, but still resulted in a 25% loss to follow up rate. This 25% is deemed 'at random' and the children of this group show similar baseline characteristics as the study population. A nonresponse bias could be expected from the parents that were contacted but did not return the questionnaires or did not consent with participation, with for example less parents responding with children experiencing difficulty in (school) development. However, all these parents were contacted by phone by the same researchers and based on these personal interactions, there was no reason to suspect such influence. From the parents that returned the CBCL forms, over half of them (45% of mothers, 48% of fathers) completed higher education, which is higher in comparison with the Dutch adult population (40% in 2019).[18] Response rates are known to be strongly and positively correlated with higher cognitive test scores at every age.[20] As the level of parental education is a major contributor of child cognitive development[21,22], our data could be skewed by the relatively high response rate among higher educated parents.

Based on our data from a homogenous treated and large cohort, we positively conclude that severe HDFN treated with IUT(s) is not associated with negative effects on children's academic and behavioural development. The conclusion is limited by the nature of our study, as survey studies are prone to various forms of bias such as response and nonresponse bias. Parents may for example be prone to give more positive evaluations of the behaviour and school performance of their children, giving rise to response bias, although we did not find major discrepancies between questionnaires and school results as reported by parents, teachers or objective school result forms.

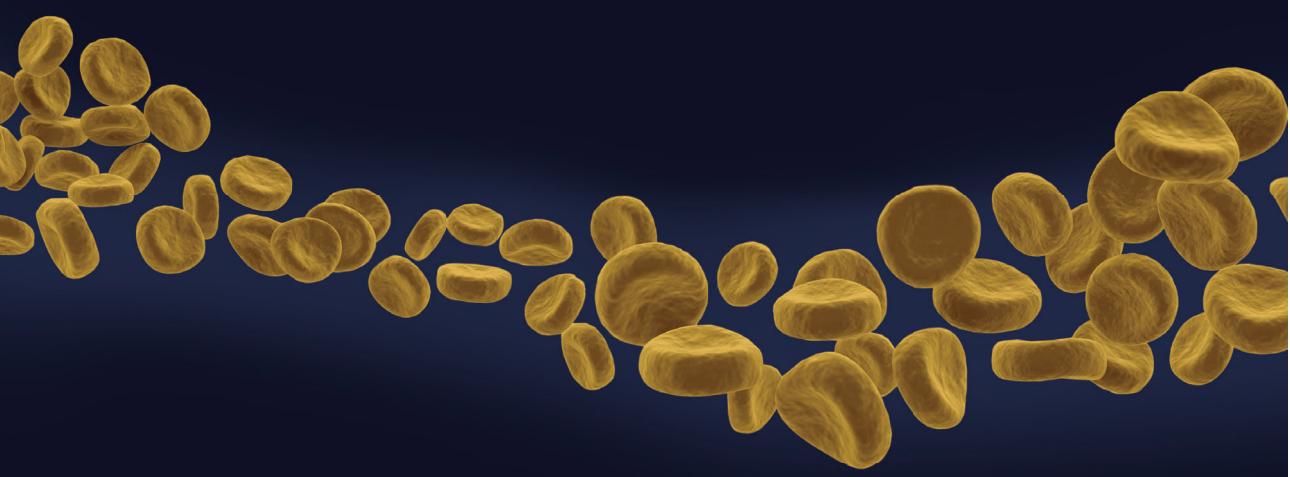
This study gives more insight in the long-term development of children with HDFN treated with IUTs and overall shows favourable and reassuring school development in children treated in an experienced foetal-therapy centre. In general, a normal distribution in school and behavioural development is to be expected for live-born children with this condition.

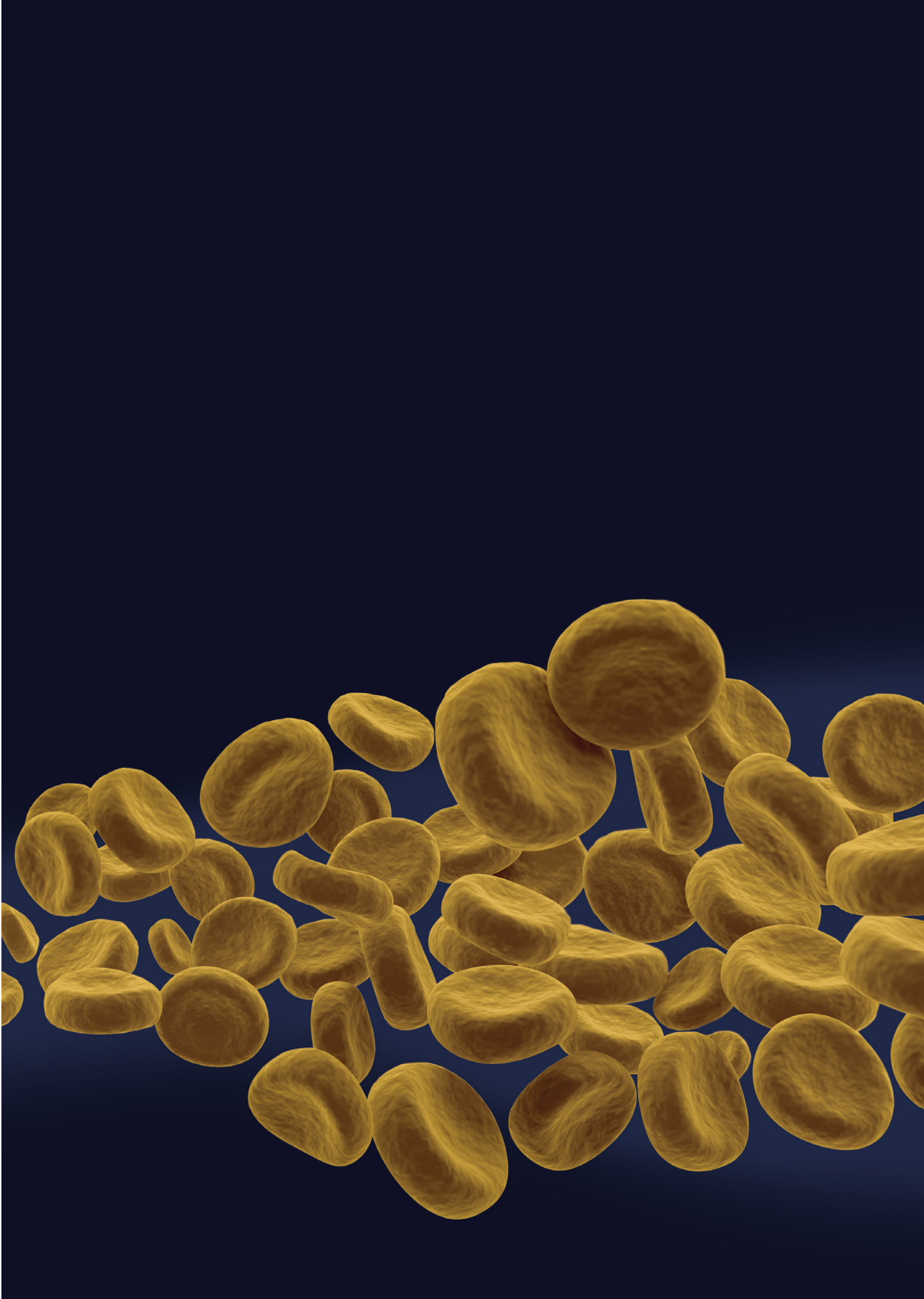
References

1. Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease, Expert Rev Hematol 2017;10(7):607-616.
2. Delaney M, Matthews DC, Hemolytic disease of the fetus and newborn: managing the mother, fetus, and newborn. Hematology Am Soc Hematol Educ Program 2015;2015:146-151.
3. Koelewijn JM, de Haas M, Vrijkotte TG, Bonsel GJ, van der Schoot CE. One single dose of 200 microg of antenatal RhIG halves the risk of anti-D immunization and hemolytic disease of the fetus and newborn in the next pregnancy. Transfusion 2008;48:1721-1729.
4. Zwiers C, Lindenburg ITM, Klumper FJ, de Haas M, Oepkes D, Van Kamp IL. Complications of intrauterine intravascular blood transfusion: lessons learned after 1678 procedures. Ultrasound Obstet Gynecol 2017;50(2):180-186.
5. Doyle LW, Kelly EA, Rickards AL, Ford GW, Callanan C. Sensorineural outcome at 2 years for survivors of erythroblastosis treated with fetal intravascular transfusions. Obstet Obstet Gynecol 1993;81(6):931-935.
6. Farrant B, Battin M, Roberts A. Outcome of infants receiving in-utero transfusions for haemolytic disease. N Z Med J 2001;114:400-403.
7. Grab D, Paulus WE, Bommer A, Buck G, Terinde R. Treatment of fetal erythroblastosis by intravascular transfusions: outcome at 6 years. Obstet Gynecol 1999;93:165-168.
8. Harper DC, Swingle HM, Weiner CP, Bonthius DJ, Aylward GP, Widness JA. Long-term neurodevelopmental outcome and brain volume after treatment for hydrops fetalis by in utero intravascular transfusion. Am J Obstet Gynecol 2006;195:192-200.
9. Hudon L, Moise KJ, Hegemier SE, Hill RM, Moise AA, Smith EO. Long-term neurodevelopmental outcome after intrauterine transfusion for the treatment of fetal hemolytic disease. Am J Obstet Gynecol 1998;179:858-863.
10. Janssens HM, de Haan MJ, Van Kamp IL, Brand R, Kanhai HH, Veen S. outcome for children treated with fetal intravascular transfusions because of severe blood group antagonism. J Pediatr 1997;131:373-380.
11. Weisz B, Rosenbaum O, Chayen B, Pelts R, Feldman B, Lipitz S. Outcome of severely anaemic fetuses treated by intrauterine transfusions. Arch Dis Child Fetal Neonatal Ed 2009;94:F201-204.
12. Lindenburg ITM, Smits-Wintjens VEHJ, van Klink JMM, Verduin E, van Kamp IL, Walther FJ, Schonewille H, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am J Obstet Gynecol 2012;206:141e1-8.
13. Achenbach TM, Ruffle TM. The Child Behavior Checklist and related forms for assessing behavioral/emotional problems and competencies. Pediatr Rev 2000;21(8):265-271.
14. Verhulst FC, van der Ende J, Koot HM. Child Behavior Checklist (CBCL)/4-18 Manual. Rotterdam: Afdeling Kinder- en Jeugdpsychiatrie, Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam/Erasmus Universiteit Rotterdam, 1996.
15. CBS. Leerlingen in (speciaal) basisonderwijs; migratieachtergrond, woonregio. [Internet]. Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/83295NED/table?ts=1597070165374 [Accessed August 10, 2020].
16. DUO. Verblijfsduur in het basisonderwijs. [Internet]. Available from: https://onderwijsincijfers.nl/kengetallen/po/leerlingen-po/prestaties-verblijfsduur [Accessed August 10, 2020].

17. Hollenberg J, van der Lubbe M. Toetsen op school. Primair onderwijs. Arnhem: Cito Corporate, 2012.
18. Maslowski R. Onderwijs. In: De sociale staat van Nederland: 2020. Available from: https://digitaal.scp.nl/ssn2020/onderwijs [Accessed February 2, 2021].
19. Hopkins-Golightly T, Raz S, Sander CJ. Influence of slight to moderate risk for birth hypoxia on acquisition of cognitive and language function in the preterm infant: a cross-sectional comparison with preterm-birth controls. Neuropsychology 2003;17(1):3-13.
20. Nishiwaki Y, Clark H, Morton SM, Leon DA. Early life factors, childhood cognition and postal questionnaire response rate in middle age: the Aberdeen Children of the 1950s study. BMC Med Res Methodol 2005;5:16.
21. Weisglas-Kuperus N, Hille ET, Duivenvoorden HJ, Finken MJJ, Wit JM, van Buuren S, van Goudoever JB, et al. Intelligence of very preterm or very low birthweight infants in young adulthood. Arch Dis Child Fetal Neonatal Ed 2009;94(3):F196-200.
22. Weisglas-Kuperus N, Baerts W, Smrkovsky M, Sauer PJ. Effects of biological and social factors on the cognitive development of very low birth weight children. Pediatrics 1993;92(5):658-665.

Part 5 - Summary and discussion





Summary

Haemolytic disease of the foetus and newborn (HDFN) is a condition in which the red blood cells of the foetus and the newborn child are destructed due to maternal alloantibodies, a process named haemolysis. Haemolysis in HDFN is caused by antibodies formed by the maternal immune system in response to contact with foreign blood group antigens, which can occur due to blood transfusion, or due to foetal-maternal transfusion during pregnancy. Maternal antibodies of the IgG class are actively transported over the placenta and if these reach the foetal circulation, haemolysis can occur if the foetal red blood cells express the recognised blood group antigens. If maternal alloantibodies reach sufficient high levels in the foetal circulation and if the antibodies are biologically active, anaemia can develop already in early pregnancy. In case of severe anaemia, it can be necessary to perform one or more blood transfusions to the anaemic foetus, so called intrauterine transfusions (IUTs).

After birth, a major complication of HDFN is hyperbilirubinaemia. Bilirubin is a waste product of haemoglobulin which is released from the destructed red blood cells and can cause neurological damage if it is present in excessive amounts. The first step in the treatment of hyperbilirubinaemia is intensive phototherapy. Phototherapy transfers bilirubin to a water-soluble form which can be more easily excreted by the kidneys and stool. If the level of bilirubin does not decrease rapidly enough under phototherapy, an exchange transfusion is the next step to remove excessive bilirubin from the circulation. During an exchange transfusion, the blood of the child is replaced with donor blood. Exchange transfusion gives a rapid decrease in bilirubin, but is also a complex procedure with potential complications.

The anaemia that can occur during pregnancy due to HDFN, can also continue weeks to months after birth. Although the connection between the foetal and maternal circulation is lost after birth, there is only a gradual decline in maternal antibodies in the child and therefore these antibodies continue to cause haemolysis for some time. In addition to continued haemolysis, anaemia in HDFN after birth is also caused and prolonged by a transfusion-induced disturbance of the physiological compensatory capacity of the bone marrow in response to anaemia. Despite a distinct decline in red blood cells, the bone marrow fails to adequately regenerate new red blood cells in response. The majority of children with severe HDFN therefore needs one or more red blood cell transfusions in the first three months after birth.

This thesis outlines the course and outcomes of HDFN after birth and provides starting points to further individualise the treatment of HDFN.

General

In **Chapter 1** an overview is given of the current management of HDFN and the available therapeutic options. The two cornerstones of treatment after birth are firstly the treatment of hyperbilirubinaemia, and therefore the prevention of neurological damage, and secondly, timely recognition of anaemia and treatment with one or more red blood cell transfusions during the first three months after birth. This chapter reviews the literature on well-known therapies such as intensive phototherapy, exchange transfusions and red blood cell transfusions, but also discusses current evidence for alternative therapies, such as treatment with exogenous erythropoietin (EPO) to treat anaemia.

Predictors of severe disease

Chapter 2 contributes to the early identification of children with a high risk for severe HDFN. In the foetus and after birth, children affected by HDFN have excessive bilirubin accumulation in the circulation compared to healthy foetuses and children. Before birth, a part of this excessive bilirubin is transported to the maternal circulation via the placenta and is therefore deemed less harmful than in the newborn child. However, it is unclear whether the levels of bilirubin that are measured in the foetus are indeed harmless and how these vary between foetuses affected by HDFN. In this study, the foetal bilirubin levels were assessed, as measured before the administration of an IUT. This study showed that these foetal bilirubin measures are of predictive value for the treatment course after birth, as high foetal bilirubin values are associated with a higher risk for treatment with exchange transfusion. Such predictors can help parents and caregivers to anticipate for severe illness after birth.

In **Chapter 3** the effect of IUT on the foetal and neonatal erythropoiesis was assessed by measuring the foetal and neonatal reticulocytes and by evaluating the need for top-op transfusions after birth. Normally, when the red blood cell count declines, the bone marrow is stimulated by feedback systems to generate more reticulocytes (immature red blood cells) to compensate and prevent anaemia. Erythropoietin is important in this process. In HDFN the destruction of red blood cells can outrun the compensatory capacity of the bone morrow, leading to anaemia which makes it necessary to perform one or more IUTs during pregnancy. This study shows that IUTs, despite the necessity for the immediate treatment of anaemia, disturb the compensatory mechanism of the bone marrow. After an IUT, an exponential decline in reticulocyte formation is observed. Children that were treated with IUTs during pregnancy, need more blood transfusions after birth and are longer transfusion-dependent compared to children not treated with IUTs.

Neonatal treatment and complications

As described in Chapter 1, the primary aim of treatment in HDFN is the treatment of hyperbilirubinaemia after birth with phototherapy and, if necessary, exchange transfusion. **Chapter 4** gives an overview of treatment with exchange transfusion in children with HDFN in the LUMC, of the last twenty years. The use of exchange transfusion has lowered considerably during this time due to improvement of care. The percentage of children with HDFN that needed one or more exchange transfusions has decreased from 67% to 10%, without an increase in complications.

Neonatal care also includes the management of postnatal anaemia. **Chapter 5** describes how 88% of children affected by HDFN that were treated with one or more IUTs, are also in need of one or more blood transfusions after birth. Of children with HDFN that did not require IUT treatment during pregnancy, 60% is in need of one or more blood transfusions after birth. To detect anaemia timely, the haemoglobin levels of all children affected by HDFN are assessed weekly during the first three months after birth. This study contributes to the identification of so called 'predictors', which might prevent unnecessary and intensive follow-up measures in the future. These specific characteristics can potentially be used to create individual risk profiles in order to fine-tune and individualise postnatal care.

As described under Chapter 5, anaemia is a complicated aspect of HDFN, with a long period of monitoring after birth and usually one or multiple readmissions to administer blood transfusions. **Chapter 6** describes the protocol of a randomised controlled trial conducted at the LUMC, which aims to evaluate the effect of exogenous erythropoietin (EPO) on the prevention of postnatal anaemia in HDFN, as potential alternative for red blood cell transfusions. Erythropoietin is a naturally occurring hormone that in the body stimulates the bone marrow to produce red blood cells. In this study, 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 darbepoetin alfa (Aranesp®, a form of erythropoietin). The hypothesis is that children treated with EPO will need less red blood cell transfusions during the first three months after birth, compared to children in the control group. This study will clarify whether EPO should be part of the standard management of HDFN to prevent anaemia after birth.

Necrotising enterocolitis (NEC) is a severe gastrointestinal condition that can occur in newborns. Although the risk to develop NEC is typically the highest among premature children

and children with (very) low birth weights, there is also a potential association between HDFN and the occurrence of NEC. **Chapter 7** reports on the occurrence of NEC among children with HDFN in the LUMC, as compared to admitted children without HDFN and gives details of the affected children and the disease course. Whereas only 1.3% (4/317) of children with HDFN developed NEC, a mere 0.4% (11/2967) of children without HDFN developed NEC. HDFN is therefore a possible distinct risk factor for NEC. Caregivers should be aware of this risk, especially in children with other risk factors such as prematurity and a low birth weight.

Long-term outcomes

Chapter 8 finally describes the long-term outcomes of children treated during pregnancy with one or more IUTs due to HDFN. Of these children between the age of 6 and 12 years, academic and behavioural functioning were assessed by evaluation of school performance reports, school progress data (grade repetition, special-needs education or additional support in school) and behavioural functioning as reported by parents and caregivers or teachers, in comparison with the Dutch norm data. The returned questionnaires and school progress data, showed favourable and reassuring outcomes regarding academic and behavioural functioning after treatment with IUTs. School performance levels for reading comprehension, spelling and mathematics showed a normal distribution and were similar for the study population and Dutch norm data, performance levels were even higher for mathematics compared to norm data. In behavioural functioning, as reported by parents and teachers, a similar incidence was observed of behavioural difficulties in children with HDFN and the norm data. In this study cohort no children attend special-needs education, as opposed to 2.6% in the Dutch norm population. In this cohort 16 children (23%) received or receive additional support in school in the form of speech therapy, support for dyslexia, or physical therapy.

Conclusion

Due to improved prevention and an efficient national screenings program, HDFN is an increasingly rare disease. This thesis evaluates the current therapy of exchange transfusion for hyperbilirubinaemia (Chapter 4) and describes exogenous erythropoietin as potential new therapeutic agent to treat anaemia (Chapter 6). It also gives starting points to individualise the treatment of these children in the future, as predictive values were identified for a more severe neonatal disease course, complicated with exchange transfusion(s) and/or red blood cell transfusion(s). Predictive values outlined in this thesis are high foetal bilirubin values (Chapter 2) and treatment with multiple IUTs during pregnancy (Chapter 3 and 5). In addition to short-term outcomes measures after birth, the long-term effects of IUTs were also critically evaluated (Chapter 8).

Nederlandse samenvatting

Hemolytische ziekte van de foetus en pasgeborene (HZFP) is een aandoening waarbij de rode bloedcellen van de foetus en het pasgeboren kind worden afgebroken door antilichamen afkomstig van de moeder, dit proces van bloedcelafbraak heet hemolyse. Hemolyse bij HZFP wordt veroorzaakt door antilichamen die het maternale immuunsysteem maakt als reactie op contact met lichaamsvreemde bloedgroepantigenen, wat kan plaatsvinden door een bloedtransfusie of transfusie tussen moeder en kind tijdens een zwangerschap. Maternale antilichamen van het IgG type worden actief getransporteerd over de placenta en als deze in de foetale bloedsomloop terechtkomen, kan er hemolyse optreden als de foetale rode bloedcellen de bloedgroepantigenen hebben die door de antilichamen herkend worden. Dit kan al in de vroege fase van een zwangerschap leiden tot ernstige bloedarmoede bij de foetus. Het kan dan nodig zijn om al tijdens de zwangerschap één of meerdere bloedtransfusies te geven, ook wel intrauteriene transfusies (IUT's).

Na de geboorte is hyperbilirubinemie de belangrijkste complicatie van HZFP. Bilirubine is een afvalproduct van hemoglobine afkomstig uit afgebroken rode bloedcellen en kan in te hoge concentraties neurologische schade veroorzaken. De eerste stap in de behandeling van hyperbilirubinemie is het geven van intensieve fototherapie. Fototherapie zorgt voor omzetting van bilirubine naar een vorm die het lichaam makkelijker kan uitscheiden. Wanneer fototherapie niet voldoende daling van het bilirubine geeft, is de volgende behandelstap om een wisseltransfusie te geven, waarbij het bloed van het kind wordt vervangen met donorbloed. Wisseltransfusie geeft een snelle daling van het bilirubine, maar is ook een gecompliceerde ingreep met kans op complicaties.

De bloedarmoede die door dit ziektebeeld ontstaat tijdens de zwangerschap, kan ook na de geboorte nog enkele weken tot maanden aanhouden. De antilichamen van moeder die de afbraak of hemolyse van bloedcellen van het pasgeboren kind veroorzaken, worden namelijk maar geleidelijk afgebroken. Daarnaast kan de balans verstoord zijn geraakt tussen afbraak van rode bloedcellen enerzijds en aanmaak van nieuwe rode bloedcellen ter compensatie door het beenmerg anderzijds. Het merendeel van deze kinderen heeft in de eerste drie maanden na de geboorte dan ook nog één of meerdere bloedtransfusies nodig.

Dit proefschrift geen een overzicht van het beloop en uitkomsten van HZFP na de geboorte en geeft handvaten om de behandeling van HZFP verder te individualiseren.

Algemeen

In **Hoofdstuk 1** wordt een overzicht gegeven van de neonatale behandelmogelijkheden bij HZFP. De twee hoekstenen van behandeling na de geboorte zijn in eerste instantie het behandelen van hyperbilirubinemie en daarmee het voorkomen van neurologische schade en daarnaast het ondervangen van bloedarmoede die kan optreden tot drie maanden na de geboorte. Dit hoofdstuk behandelt de literatuur over gevestigde therapieën als intensieve fototherapie, wisseltransfusie en bloedtransfusies, maar behandelt ook de stand van zaken betreffende alternatieve behandelingen zoals het toedienen van exogeen erytropoëtine (EPO) ter behandeling van bloedarmoede.

Voorspellers van ernstige ziekte

Hoofdstuk 2 draagt bij aan het vroegtijdig identificeren van kinderen met een hoger risico voor een ernstig ziektebeloop. Zowel foetaal als na de geboorte komt meer bilirubine vrij bij dit ziekteproces dan in gezonde foetussen en kinderen. Voor de geboorte wordt een deel van het excessieve bilirubine via de placenta naar de maternale circulatie afgevoerd, maar het is onduidelijk of de bij de foetus gemeten waarden van bilirubine al schade kunnen aanrichten. In deze studie is gekeken naar de hoogte van de foetale bilirubinewaardes, zoals gemeten voor het toedienen van een IUT. Daarnaast is aangetoond dat deze foetaal gemeten waardes, voorspellend zijn voor intensieve behandeling na de geboorte in de vorm van een wisseltransfusie. Ouders en zorgverleners kunnen hierdoor al voor de geboorte beter voorbereid worden op een mogelijk ernstig neonataal beloop.

In **Hoofdstuk 3** is gekeken naar het effect van IUT's op de foetale en neonatale erythropoiese (bloedsaanmaak) door de foetale en neonatale reticulocyten te meten. Reticulocyten zijn jonge voorlopercellen van rode bloedcellen. In het geval van dreigende bloedarmoede, zal het beenmerg door terugkoppeling meer jonge rode bloedcellen, reticulocyten, aanmaken ter compensatie. De stof erytropoëtine is belangrijk in dit proces. In HZFP gaat de afbraak van rode bloedcellen veelal sneller dan het beenmerg in staat is te compenseren, waardoor één of meerdere IUT's nodig kunnen zijn tijdens de zwangerschap om bloedarmoede te bestrijden. Deze studie laat zien dat IUT's, ondanks noodzakelijk voor de acute bestrijding van bloedarmoede, het compensatiemechanisme van het beenmerg verstoren en dat na een IUT een exponentiële daling in de aanmaak van reticulocyten plaatsvindt. Kinderen die tijdens de zwangerschap behandeld zijn met IUT's, hebben dan ook vaker en langer bloedtransfusies nodig na de geboorte dan kinderen die niet behandeld zijn met IUT's.

Neonatale behandeling en complicaties

Als beschreven in Hoofdstuk 1, is de behandeling van HZFP sterk gericht op het behandelen van hyperbilirubinemie na de geboorte middels fototherapie en zo nodig wisseltransfusie. **Hoofdstuk 4** geeft een overzicht van het gebruik van wisseltransfusies bij kinderen met HZFP in het LUMC van de afgelopen twintig jaar. Daaruit blijkt dat wisseltransfusies tegenwoordig veel minder vaak nodig zijn door verbeteringen in de zorg, het gaat om een daling van 67% van de kinderen met HZFP naar 10% de afgelopen vijf jaar, zonder toename in complicaties.

Naast het behandelen van hyperbilirubinemie, is behandeling ook gericht op het ondervangen van bloedarmoede. In **Hoofdstuk 5** wordt beschreven dat tot 88% van de kinderen met HZFP die tijdens de zwangerschap behandeld zijn met één of meerdere IUT's, ook na de geboorte één of meerdere bloedtransfusies nodig hebben. Kinderen met HZFP die tijdens de zwangerschap geen IUT nodig hadden, komen in 60% van de gevallen toe aan één of meerdere bloedtransfusies na de geboorte. Om bloedarmoede op te sporen wordt op dit moment bij alle kinderen met HZFP tot de leeftijd van drie maanden de bloedwaarden wekelijks gecontroleerd. Deze studie draagt bij aan het identificeren van zogenoemde 'voorspellers', waardoor het wellicht in de toekomst niet meer nodig is om alle kinderen zo vaak bloed te laten prikken. Aan de hand van deze specifieke kenmerken kan een individueel risicoprofiel per kind worden gemaakt ter betere afstemming van de nazorg.

Zoals beschreven bij Hoofdstuk 5, is bloedarmoede een belastend aspect van HZFP, waarbij langdurige bloedcontroles nodig zijn en veelal meerdere heropnames geïndiceerd zijn voor het toedienen van bloedtransfusies. **Hoofdstuk 6** beschrijft het protocol van een gerandomiseerd onderzoek uitgevoerd vanuit het LUMC naar het effect van exogeen erytropoëtine (EPO) op het voorkomen van bloedarmoede bij deze kinderen, als potentieel alternatief voor bloedtransfusies. Erytropoëtine is een natuurlijk voorkomend hormoon dat in ons lichaam in het beenmerg de productie van rode bloedcellen stimuleert. Tijdens de studieperiode worden kinderen geloot tussen conventionele behandeling (controlegroep met wekelijkse bloedcontroles en indien nodig een bloedtransfusie) en aanvulling hiervan met wekelijkse thuisbezoeken en toediening van het medicament darbepoëtine alfa (Aranesp®, een vorm van erytropoëtine). De hypothese is dat kinderen die behandeld worden met EPO, minder vaak een bloedtransfusie nodig hebben in de eerste drie maanden na geboorte dan kinderen in de controlegroep. Deze studie zal duidelijk maken of er een plaats is voor EPO in de standaard behandeling van kinderen met HZFP ter voorkoming van bloedarmoede na de geboorte.

Necrotiserende enterocolitis (NEC) is een ernstige darmaandoening die kan optreden bij pasgeboren kinderen. Hoewel met name te vroeg geboren kinderen en kinderen met een laag geboortegewicht een risico lopen om NEC te ontwikkelen, bestaat mogelijk ook een associatie tussen HZFP en het optreden van NEC. In **Hoofdstuk 7** is nagegaan of NEC vaker voorkomt bij kinderen met HZFP in het LUMC, vergeleken met opgenomen kinderen zonder HZFP en zijn de details van aangedane kinderen en hun ziekteverloop beschreven. Hoewel slechts 1,3% (4/317) van de kinderen met HZFP deze complicatie ontwikkelden, was van de kinderen zonder HZFP slechts 0,4% (11/2967) van de kinderen aangedaan. HZFP is daarmee mogelijk een aparte risicofactor voor het ontwikkelen van NEC, waar zorgverleners zich bewust van moeten zijn, zeker in combinatie met andere risicofactoren zoals prematuriteit en een laag geboortegewicht.

Langetermijnuitkomsten

Hoofdstuk 8 beschrijft tot slot de langetermijnuitkomsten van kinderen die tijdens de zwangerschap één of meer IUT's hebben ondergaan vanwege HZFP. Van deze kinderen die nu tussen de 6 en 12 jaar zijn, zijn het schoolpresteren en gedragsmatig functioneren in kaart gebracht door schoolresultaten van de Cito-toetsen, schoolvoortgangsgegevens (doubleren, speciaal onderwijs of aanvullende ondersteuning) en gedragsmatig functioneren als gerapporteerd door ouders of verzorgers en leerkrachten te vergelijken met de Nederlands normpopulatie. De teruggestuurde vragenlijsten en schoolvoortgangsgegevens, toonden een positief en geruststellend beeld van het schoolpresteren en gedragsmatig functioneren van kinderen die aan IUT's zijn blootgesteld tijdens de zwangerschap met een normale verdeling in Cito-toets uitslagen op het gebied van begrijpend lezen en spelling, vergelijkbaar met die van de Nederlandse normpopulatie. Cito-scores voor het onderdeel wiskunde waren zelfs hoger dan van de normpopulatie. Ook in gedragsmatig functioneren, als gerapporteerd door ouders en leerkrachten, werd een gelijke incidentie gezien van gedragsproblemen in kinderen met HZFP en de controlepopulatie. In de studiegroep waren geen kinderen die speciaal onderwijs volgen, terwijl 2,6% van de Nederlandse jeugd speciaal onderwijs volgt. In de studie waren 16 kinderen (23%) die aanvullende ondersteuning op school kregen of krijgen in de vorm van logopedie, ondersteuning voor dyslexie of fysiotherapie.

Conclusie

Door verbeterde preventie en een efficiënt nationaal screeningsprogramma, is HZFP een toenemend zeldzaam ziektebeeld. Dit proefschrift evalueert de gevestigde therapie van wisseltransfusie voor hyperbilirubinemie (Hoofdstuk 4) en beschrijft een potentiële nieuwe behandelmogelijkheid voor bloedarmoede in de vorm van exogeen erytropoëtine (Hoofdstuk 6). Daarnaast biedt het handvaten om in de toekomst de behandeling van deze kinderen verder te individualiseren, waarbij onder meer factoren als hoge foetale bilirubine waardes (Hoofdstuk 2) en behandeling met meerdere IUT's tijdens de zwangerschap (Hoofdstuk 3 en 5) als voorspellende factoren zijn geïdentificeerd voor een ernstiger neonataal beloop met wisseltransfusie en/of bloedtransfusies. Naast het beloop direct na de geboorte, zijn ook de langetermijnuitkomsten van IUT's kritisch geëvalueerd (Hoofdstuk 8).

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.

References

1. Bowman J. Thirty-five years of Rh prophylaxis. Transfusion 2003;43:1661-1666.
2. Zwiers C, Oepkes D, Lopriore E, Klumper FJ, de Haas M, van Kamp IL. The near disappearance of fetal hydrops in relation to current state-of-the-art management of red cell alloimmunization. Prenat Diagn 2018;38(12):943-950.
3. Zwiers C, Lindenburg ITM, Klumper FJ, de Haas M, Oepkes D, Van Kamp IL. Complications of intrauterine intravascular blood transfusion: lessons learned after 1678 procedures. Ultrasound Obstet Gynecol 2017;50(2):180-186.
4. Ree IMC, Besuden CFJ, Zwiers C, Verweij EJT, Oepkes D, de Haas M, Lopriore E. Exchange transfusions in severe alloimmune hemolytic disease of the fetus and newborn: a 20-year overview on the incidence, associated risks and outcome. Vox Sang 2021;116(9):990-997.
5. Ree IMC, van 't Oever RM, Jansen L, Lopriore E, de Haas M, van Klink JMM. School performance and behavioral functioning in children after intrauterine transfusions for hemolytic disease of the fetus and newborn. Early Hum Dev 2021;157:105381.
6. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. Vox Sang 2015;109:99-113.
7. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev 2000;14:44-61.
8. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med 2008;13:207-214.
9. Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. Expert Rev Hematol 2017;10(7):607-616.
10. Millard DD, Gidding SS, Socol ML, MacGregor SN, Dooley SL, Ney JA, Stockman JA. Effects of intravascular, intrauterine transfusion on prenatal and postnatal hemolysis and erythropoiesis in severe fetal isoimmunization. J Pediatr 1990;117(3):447-454.
11. De Boer IP, Zeestraten EC, Lopriore E, van Kamp IL, Kanhai HH, Walther FJ. Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008;198(1):51-54.
12. Ree IMC, Middelburg RA, Zwiers C, Oepkes D, van der Bom JG, Lopriore E. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth. Br J Haematol 2019;186(4):565-573.
13. Ree IMC, Lopriore E, Zwiers C, Böhringer S, Janssen MWM, Oepkes D, De Haas M. Suppression of compensatory erythropoiesis in hemolytic disease of the fetus and newborn due to intrauterine transfusions. Am J Obstet Gynecol 2020;223(1):119.e1-119.e10.
14. Bhutani VK, Johnson-Hamerman L. The clinical syndrome of bilirubin-induced neurologic dysfunction. Semin Fetal Neonatal Med 2015;20:6-13.
15. Schenker S, Dawber NH, Schmid R. Bilirubin Metabolism in the Fetus. J Clin Invest 1964;43:32-39.
16. Lester R, Behrman RE, Lucey IF. Transfer of Bilirubin-C14 Across Monkey Placenta. Pediatrics 1963;32:416-419.
17. Serrano MA, Bayón JE, Pascolo L, Tiribelli C, Ostrow JD, Gonzalez-Gallego J, Marin JJG. Evidence for carrier-mediated transport of unconjugated bilirubin across plasma membrane vesicles from human placental trophoblast. Placenta 2002;23(7):527-535.

18. Ree IMC, van 't Oever RM, Zwiers C, Verweij EJT, Oepkes D, de Haas M, Loprlore E. Are fetal bilirubin levels associated with the need for neonatal exchange transfusions in hemolytic disease of the fetus and newborn? Am J Obstet Gynecol MFM 2021;18;3(3):100332.
19. Maisels MJ, McDonagh AF: Phototherapy for neonatal jaundice. N Engl J Med 2008; 358:920-928.
20. American Academy of Pediatrics Subcommittee on H. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316.
21. Chhapola V, Sharma AG, Kanwal SK, Kumar V, Patra B. Neonatal exchange transfusions at a tertiary care centre in north India: an investigation of historical trends using change-point analysis and statistical process control. Int Health 2018;10(6):451-456.
22. Huizing K, Roislien J, Hansen, T. Intravenous immune globulin reduces the need for exchange transfusions in Rhesus and AB0 incompatibility. Acta Paediatr 2008;97(10):1362-1365.
23. Steiner LA, Bizzarro MJ, Ehrenkranz RA, Gallagher PG. A decline in the frequency of neonatal exchange transfusions and its effect on exchange-related morbidity and mortality. Pediatrics 2007;120(1):27-32.
24. al-Alaiyan S, al Omran A. Late hyporegenerative anaemia in neonates with rhesus haemolytic disease. J Perinat Med 1999;27:112-115.
25. Thorp JA, O'Connor T, Callenbach J, Cohen GR, Yeast JD, Albin J, Plapp F. Hyporegenerative anemia associated with intrauterine transfusion in rhesus hemolytic disease. Am J Obstet Gynecol 1991;165:79-81.
26. Nicaise C, Gire C, Casha P, d'Ercole C, Chau C, Palix C. Erythropoietin as treatment for late hyporegenerative anemia in neonates with Rh hemolytic disease after in utero exchange transfusion. Fetal Diagn Ther 2002;17:22-24.
27. Ohls RK, Wirkus PE, Christensen RD. Recombinant erythropoietin as treatment for the late hyporegenerative anemia of Rh hemolytic disease. Pediatrics 1992;90:678-680.
28. Ovali F, Samanci N, Dagoglu T. Management of late anemia in Rhesus hemolytic disease: use of recombinant human erythropoietin (a pilot study). Pediatr Res 1996;39:831-834.
29. Scaradavou A, Inglis S, Peterson P, Dunne J, Chervenak F, Bussel J. Suppression of erythropoiesis by intrauterine transfusions in hemolytic disease of the newborn: use of erythropoietin to treat the late anemia. J Pediatr 1993;123:279-284.
30. Zuppa AA, Alighieri G, Calabrese V, Visintini F, Cota F, Carducci C, Antichi E, et al. Recombinant human erythropoietin in the prevention of late anemia in intrauterine transfused neonates with Rh-isoimmunization. J Pediatr Hematol Oncol 2010;32:e95-101.
31. Zuppa AA, Maragliano G, Scapillati ME, Florio MG, Girlando P, Noia G, de Santis M, et al. Recombinant erythropoietin in the prevention of late anaemia in intrauterine transfused neonates with Rh-haemolytic disease. Fetal Diagn Ther 1999;14:270-274.
32. Aher S, Ohlsson A. Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2006;3:CD004868.
33. Ohls RK, Kamath-Rayne BD, Christensen RD, Wiedmeier SE, Rosenberg A, Fuller J, Lacy CB, et al. Cognitive outcomes of preterm infants randomized to darbepoetin, erythropoietin, or placebo. Pediatrics 2014;133(6):1023-1030.
34. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev 2006;3:CD004863.
35. Dallacasa P, Ancora G, Miniero R, Gavella B, Brondelli L, Conte R, Salvioli GP. Erythropoietin course

in newborns with Rh hemolytic disease transfused and not transfused in utero. Pediatr Res 1996;40:357-360.

36. Rath ME, Smits-Wintjens VE, Oepkes D, van Zwet EW, van Kamp IL, Brand A, Walther FJ, et al. Thrombocytopenia at birth in neonates with red cell alloimmune haemolytic disease. Vox Sang 2012;102:228-233.
37. Smits-Wintjens VEHJ, Rath ME, Lindenburg IT, Oepkes D, van Zwet EW, Walther FJ, Lopriore E. Cholestasis in neonates with red cell alloimmune hemolytic disease: incidence, risk factors and outcome. Neonatology 2012;101:306-10.
38. Rao SC, Patole SK, Dickinson JE, Reid KP, Doherty DA. Neonatal necrotising enterocolitis following intrauterine transfusions: is there an association? J Matern Fetal Neonatal Med 2004;16(1):51-54.
39. Roig JC, Burchfield DJ. Term neonates with haemolytic disease of the newborn and necrotising enterocolitis: a report of two cases. J Perinatol 1994;14(3):201-203.
40. Dominguez KM, Moss RL. Necrotising enterocolitis. Clin Perinatol 2012;39(2):387-401.
41. Lambert DK, Christensen RD, Henry E, Besner GE, Baer VL, Wiedmeier SE, Stoddard RA, et al. Necrotising enterocolitis in term neonates: data from a multihospital health-care system. J Perinatol 2007;27(7):437-443.
42. Lin PW and Stoll BJ. Necrotising enterocolitis. Lancet 2006;368(9543):1271-1283.
43. Ree IMC, de Grauw AM, Bekker V, de Haas M, te Pas A, Oepkes D, Middeldorp JM, et al. Necrotising enterocolitis in haemolytic disease of the newborn: a retrospective cohort study. Vox Sang 2020;115(2):196-201.
44. Doyle LW, Kelly EA, Rickards AL, Ford GW, Callanan C. Sensorineural outcome at 2 years for survivors of erythroblastosis treated with fetal intravascular transfusions. Obstet Obstet Gynecol 1993;81(6):931-935.
45. Farrant B, Battin M, Roberts A. Outcome of infants receiving in-utero transfusions for haemolytic disease. N Z Med J 2001;114:400-403.
46. Grab D, Paulus WE, Bommer A, Buck G, Terinde R. Treatment of fetal erythroblastosis by intravascular transfusions: outcome at 6 years. Obstet Gynecol 1999;93:165-168.
47. Harper DC, Swingle HM, Weiner CP, Bonthius DJ, Aylward GP, Widness JA. Long-term neurodevelopmental outcome and brain volume after treatment for hydrops fetalis by in utero intravascular transfusion. Am J Obstet Gynecol 2006;195:192-200.
48. Hudon L, Moise KJ, Hegemier SE, Hill RM, Moise AA, Smith EO. Long-term neurodevelopmental outcome after intrauterine transfusion for the treatment of fetal hemolytic disease. Am J Obstet Gynecol 1998; 179: 858-863.
49. Janssens HM, de Haan MJ, van Kamp IL, Brand R, Kanhai HH, Veen S. Outcome for children treated with fetal intravascular transfusions because of severe blood group antagonism. J Pediatr 1997;131:373-380.
50. Weisz B, Rosenbaum O, Chayen B, Pelts R, Feldman B, Lipitz S. Outcome of severely anaemic fetuses treated by intrauterine transfusions. Arch Dis Child Fetal Neonatal Ed 2009;94:F201-204.
51. Lindenburg IT, Smits-Wintjens VE, van Klink JMM, Verduin E, van Kamp IL, Walther FJ, Schonewille H, et al.; LOTUS study group. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am J Obstet Gynecol 2012;206(2):141.e1-8.

52. Bhutani VK, Zipursky A, Blencowe H, Khanna R, Sgro M, Ebbesen F, Bell J, et al. Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res 2013;74(1):86-100.

53. Castleman JS, Moise KJ, Kilby MD. Medical therapy to attenuate fetal anaemia in severe maternal red cell alloimmunization. Br J Haematol 2021;192(3):425-432.

54. Peter HH, Ochs HD, Cunningham-Rundles C, Vinh DC, Kiessling P, Greve B, Jolles S. Targeting FcRn for immunomodulation: Benefits, risks, and practical considerations. J Allergy Clin Immunol 2020;146(3):479-491.

55. Ree IMC, Lopriore E. updates in neonatal hematology: causes, risk factors, and management of anemia and thrombocytopenia. Hematol Oncol Clin North Am 2019;33(3):521-532.

56. Kirpalani H, Bell EF, Hintz SR, Tan S, Schmidt B, Chaudhary AS, Johnson KJ, et al. Higher or Lower Hemoglobin Transfusion Thresholds for Preterm Infants. N Engl J Med 2020;383(27):2639-2651.

57. Bard H. The postnatal decline of hemoglobin F synthesis in normal full-term infants. J Clin Invest 1975;55(2):395-398.

58. Finne PH, Halvorsen S. Regulation of erythropoiesis in the fetus and newborn. Arch Dis Child 1972;47(255):683-687.

59. Teofili L, Papacci P, Orlando N, Bianchi M, Molisso A, Purcaro V, Valentini CG, et al. Allogeneic cord blood transfusions prevent fetal haemoglobin depletion in preterm neonates. Results of the CB-TrIP study. Br J Haematol 2020;191(2):263-268.

60. Khodabux CM, Brand A. The use of cord blood for transfusion purposes: current status. Vox Sang 2009;97(4):281-293.

61. Slootweg YM, Walg C, Koelewijn JM, Van Kamp IL, De Haas M. Knowledge, attitude and practices of obstetric care providers towards maternal red-blood-cell immunization during pregnancy. Vox Sang 2020;115(3):211-220.

Appendices

List of abbreviations

AAP	American Academy of Pediatrics
ADCC	Antibody-dependent cell-mediated cytotoxicity
AUC	Area under the curve
BIND	Bilirubin-induced neurologic dysfunction
CBCL	Child behavioural checklist
CI	Confidence interval
EPO	Erythropoietin
ET	Exchange transfusion
GA	Gestational age
HbA	Adult haemoglobin
HbF	Foetal haemoglobin
HDFN	Haemolytic disease of the foetus and newborn
IQR	Interquartile range
IUT	Intrauterine transfusions
IVH	Intraventricular haemorrhage
IVIg	Intravenous immunoglobulins
K	Kell, blood group system
LED	Light emitting diode
LUMC	Leiden University Medical Centre
MCA	Middle cerebral artery
NDI	Neurodevelopmental impairment
NEC	Necrotising enterocolitis
OR	Odds ratio
PVL	Periventricular leukomalacia
RBC	Red blood cell
RCT	Randomised controlled trial
Rh	Rhesus, blood group system
RhIg	Rhesus D immunoprophylaxis
ROC	Receiver operating curve
SD	Standard deviation
SGA	Small for gestational age
TRF	Teacher report form

List of publications

This thesis

- Ree IMC, van 't Oever RM, Jansen L, Lopriore E, de Haas M, van Klink JMM. School performance and behavioral functioning in children after intrauterine transfusions for hemolytic disease of the fetus and newborn. *Early Hum Dev* 2021;157:105381.
- Ree IMC, Besuden CFJ, Zwiers C, Verweij EJT, Oepkes D, de Haas M, Lopriore E. Exchange transfusions in severe alloimmune hemolytic disease of the fetus and newborn: a 20-year overview on the incidence, associated risks and outcome. *Vox Sang* 2021;116(9):990-997.
- Ree IMC, van 't Oever RM, Zwiers C, Verweij EJT, Oepkes D, de Haas M, Lopriore E. Are fetal bilirubin levels associated with the need for neonatal exchange transfusions in hemolytic disease of the fetus and newborn? *Am J Obstet Gynecol MFM* 2021;18;3(3):100332.
- Ree IMC, Lopriore E, Zwiers C, Böhringer S, Janssen MWM, Oepkes D, de Haas M. Suppression of compensatory erythropoiesis in hemolytic disease of the fetus and newborn due to intrauterine transfusions. *Am J Obstet Gynecol* 2020;223(1):119.e1-119.e10.
- Ree IMC*, de Grauw AM*, Bekker V, de Haas M, te Pas A, Oepkes D, Middeldorp JM, Lopriore E. Necrotising enterocolitis in haemolytic disease of the newborn: a retrospective cohort study. *Vox Sang* 2020;115(2):196-201.
- Ree IMC, Middelburg RA, Zwiers C, Oepkes D, van der Bom JG, Lopriore E. Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth. *Br J Haematol* 2019;186(4):565-573.
- Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. *Expert Rev Hematol* 2017;10(7):607-616.

*shared first authorship, authors contributed equally

Other publications

- Ree IMC, Lopriore E. Updates in Neonatal Hematology: Causes, Risk Factors, and Management of Anemia and Thrombocytopenia. *Hematol Oncol Clin North Am*. 2019;33(3):521-32.
- Fustolo-Gunnink SF, Fijnvandraat K, Putter H, Ree IMC, Caram-Deelder C, Andriessen P, d'Haens EJ, Hulzebos CV, Onland W, Kroon AA, Vijlbrief DC, Lopriore E, van der Bom JG. Dynamic prediction of bleeding risk in thrombocytopenic preterm neonates. *Haematologica* 2019;104(11):2300-6.
- Ree IMC, Fustolo-Gunnink SF, Bekker V, Fijnvandraat KJ, Steggerda SJ, Lopriore E. Thrombocytopenia in neonatal sepsis: Incidence, severity and risk factors. *PLoS One* 2017;12(10): e0185581.
- Ree IMC, Smits-Wintjens VE, Rijntjes-Jacobs EG, Pelsma IC, Steggerda SJ, Walther FJ, Lopriore E. Necrotizing enterocolitis in small-for-gestational-age neonates: a matched case-control study. *Neonatology* 2014;105(1):74-8.
- Ree IMC, Kaptein AA. "Noem het maar reuma": twee patiëntenverhalen over functionele aandoeningen. *Nederlands Tijdschrift voor Reumatologie* 2011:4;44-6.

Curriculum vitae

Isabelle Ree werd geboren op 22 augustus 1991 te Capelle aan den IJssel als middelste dochter van Han en Marjan Ree. Zij doorliep het atheneum aan het IJsselcollege te Capelle aan den IJssel en behaalde in 2009 cum laude haar eindexamen. Op 31 augustus 2009 startte zij met de studie geneeskunde aan de Universiteit Leiden en ontmoette zij haar latere echtgenoot Joost. Tijdens haar studie was zij achtereenvolgens werkzaam als bijlesdocente bij Curium centrum voor Kinder- en jeugdpsychiatrie, als verzorgende in de ouderenverzorging en was zij columniste voor de Universiteit Leiden. Haar eerste aanraking met de kindergeneeskunde was in 2012, op de afdeling neonatologie van het São João ziekenhuis in Porto, Portugal, in het kader van een klinische uitwisseling. Deze uitwisseling was aanleiding tot een wetenschappelijke stage op de afdeling kindergeneeskunde van het LUMC, alwaar zij voor het eerst in contact kwam met prof. dr. Enrico Lopriore. Na haar afstuderen in 2015, is Isabelle in 2016 begonnen met promotieonderzoek op de afdelingen kindergeneeskunde (LUMC) en klinische transfusiegeneeskunde (Sanquin), onder begeleiding van prof. dr. Enrico Lopriore en prof. dr. Masja de Haas. Het promotieonderzoek heeft zij in deeltijd afgerond, sinds 2019 was zij naast het onderzoek achtereenvolgens werkzaam als arts-assistent niet in opleiding op de afdeling kindergeneeskunde van het Haaglanden Medisch Centrum, locatie Westeinde te Den Haag, het HagaZiekenhuis te Den Haag en het Maasstad Ziekenhuis te Rotterdam.

Dankwoord

Mijn promotietraject startte in oktober 2016 en in de jaren sindsdien hebben veel mensen op verschillende wijzen een bijdrage aan dit proefschrift geleverd. Een aantal mensen hiervan wil ik in het bijzonder bedanken.

Beste Enrico en Masja, jullie zijn de yin en yang van mijn PhD-traject. De combinatie van jullie beiden en de balans tussen jullie karakters, waren onmisbaar om tot dit eindproduct te komen. Enrico, bedankt voor je humor, oneindige energie en alle kansen die je me geboden hebt in de negen jaar dat ik je al ken en de vrijheid en het vertrouwen om mijn eigen weg te kiezen. Masja, ik heb grote bewondering voor je onbedwingbare onderzoekersgeest, die verbanden legt en in het tijdsbestek van een kop koffie tientallen nieuwe onderzoeksrichtingen ziet. Het is een groot geluk om met jullie onderzoek te mogen doen, veel dank aan jullie beiden.

Beste Jeanine, je bent als co-promotor een grote steun voor mij geweest binnen het onderzoek en op persoonlijk vlak. Ik dank je voor je humor, je vertrouwen en eerlijkheid, ik zal onze gesprekken niet snel vergeten.

Binnen het onderzoeksteam heb ik jaren een kamer gedeeld met Henriëtte, Janneke, Lisanne en Vivian. De intelligentie, humor en onvoorwaardelijke steun die ik in deze kamer gevoeld heb, maken deze sterke vrouwen de schaduwauteurs van elk stuk uit dit proefschrift en ik wil ze hier dan ook graag extra bedanken. Het is gelukt, mijn proefschrift mag eindelijk naast dat van jullie staan!

Natuurlijk had ik het ook niet gekund zonder de rest van het onderzoeksteam om de hoogte- en dieptepunten van een promotietraject mee te delen. Alle dank aan Amber, Carolien, Denise, Derek, Dian, Emma, Erik, Hilda, Hylke, Kristel, Lisette, Lisanne H., Marieke, Marlies, Patricia, Romy, Sophie C., Sophie G., Sophie J., Tessa, Thijs en alle nieuwe collega's die het werk voortzetten. Mijn tweede onderzoeksfamilie bij Sanquin kan ik daarbij niet vergeten, met in het bijzonder Josine en natuurlijk ook Anske, Camila, Inge, Leo, Loes, Meryem, Rutger, Sarah, Sharon, Sufia en de rest van het team.

Daarnaast wil ik de stafleden, secretaresses, arts-assistenten en verpleegkundigen van de afdelingen neonatologie en verloskunde bedanken, die allen hun armen en geest wagenwijd open hebben staan voor onderzoek en op alle mogelijke manieren hieraan bijdragen.

Ook bedank ik de collega's en nieuwe vrienden van de afdelingen kindergeneeskunde van het Haaglanden Medisch Centrum en het HagaZiekenhuis te Den Haag en het Maasstad Ziekenhuis

te Rotterdam. Zij maakten van deze onderzoeker een klinisch dokter en boden me de ruimte om deze rollen naast elkaar te vervullen.

Mijn grote dank gaat uiteraard vooral ook uit naar de ouders en kinderen die deelnamen aan de EPO-studie. Ze hebben me alle hoeken van het land laten zien op thuisbezoek en een kijkje gegeven in de dagelijkse praktijk van dit ziektebeeld. Ze waren, elke kilometer van de 20.421 kilometer in totaal, de inspiratie die ik nodig had.

Uiteraard dank ik daarnaast alle andere lieve vrienden, familie en schoonfamilie die altijd een luisterend oor, glas wijn, of schouder om op te huilen boden. Op twee van jullie kan ik tijdens de verdediging nog extra leunen, mijn paranimfen Lisanne en Iris. Lisanne, dankzij jou weet ik dat groots zijn niets met lengte te maken heeft. En Iris, dank je voor je dinsdagen, onvoorwaardelijke steun en je eerlijkheid. Zonder je advies dat promoveren niets voor mij is, had ik hier niet gestaan.

Lieve zussen, ons ouderlijk gezin is de fundering van mijn hele wereld. Esther, ik heb vanaf de eerste rij als jij optreedt altijd gezien wat het betekent om te vechten voor je passie en nooit op te geven, je bent daarin altijd mijn grote voorbeeld geweest. Bedankt dat jij en Rik er altijd voor me zijn en dat jullie me de leukste neefjes ter wereld hebben gegeven. En Charmaine, kleintje, ik heb twee keer met mijn ogen geknipperd en nu ben je opeens een volwassen, sterke vrouw die recht op haar doel afgaat en mij met beide benen op de grond zet.

Lieve papa en mama, Han en Marjan, dit proefschrift is voor jullie. Jullie hebben altijd geloofd dat ik ooit een boek zou schrijven en hier ligt het, al is het misschien een ander boek dan verwacht. Jullie liefde en onvoorwaardelijke vertrouwen, voel ik bij elke stap die ik zet. Ik kan niet zonder jullie.

Lieve Joost, de laatste woorden van dit proefschrift zijn voor jou. Het is een voorrecht om vanaf de allereerste dag geneeskunde naast jou te mogen staan en samen met jou dokter te zijn geworden. Ook tijdens dit onderzoekstraject ben je niet van mijn zijde geweken en raapte je me van de grond, of sleepte je me aan mijn enkels verder als het moest. Jouw liefde, humor en intelligentie zijn de veilige armen waarin ik thuiskom. Dank je dat ik je computer mocht gebruiken.