Hematological outcome
in neonatal alloimmune hemolytic disease

Layout & printing: Ipskamp Drukkers B.V., Enschede, The Netherlands
Cover design: drawing by Mirjam Rath, digitally edited by José Bidegain

Financial support for the publication of this thesis was provided by the Department of Pediatrics Leiden University Medical Center (Willem-Alexander Kinderziekenhuis), Nutricia Nederland BV, Sanquin Bloedvoorziening, Abbott BV, the Department of Obstetrics and Fetal Medicine Leiden University Medical Center and Vygon BV

Hematological outcome in neonatal alloimmune hemolytic disease

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 7 maart 2013
klokke 13.45 uur

door

Mirjam Eva Aafke Rath
geboren te ’s-Gravenhage
in 1983

Promotiecommissie

Promotor:	Prof. dr. F.J. Walther
Co-promotor:	Dr. E. Lopriore
Overige leden:	Prof. dr. A. Brand
	Prof. dr. J.M.M. van Lith
	Prof. dr. D. Oepkes
	Dr. P.H. Dijk, Universitair Medisch Centrum Groningen
	Dr. K. Fijnvandraat, Academisch Medisch Centrum
	Dr. J.J. Zwaginga

Contents

Chapter 1

General introduction

General introduction

Pathophysiology

Hemolytic disease of the fetus and newborn (HDFN), in earlier times known as erythroblastosis fetalis, is a condition in which fetal and neonatal red cells are destructed by the action of IgG antibodies derived from the mother by placental transfer. Maternal alloimmunization usually occurs after fetal-maternal hemorrhage at the time of delivery or during pregnancy when fetal red cells which express paternally derived antigens enter the maternal circulation.[1] In contrast to fetal and neonatal alloimmune thrombocytopenia, first pregnancies in HDFN are usually not affected because fetomaternal hemorrhage is too small or too late to cause formation of IgG antibodies.[1,2] Maternal immunization may also result from a blood transfusion and partly for that reason primigravida can still be affected.[3]

Over 50 red blood cell antigens can cause HDFN, however only Rh D, Kell (K1) and Rh c alloimmunization are frequently associated with severe fetal disease.[4] When fetal disease is left untreated, this can lead to progressive fetal anemia, fetal hydrops and fetal death. Fetal hydrops consists of abnormal fluid accumulation in fetal compartments, including ascites, pleural effusion, pericardial effusion, and skin edema. It is assumed that immune fetal hydrops results from congestive heart failure due to severe anemia.[5-7]

After birth, placental transfer of antibodies stops, but already derived antibodies can remain in the circulation for several months. As a consequence, hemolysis continues leading to neonatal hyperbilirubinemia and anemia which can be present up to three months of age.[8] Hyperbilirubinemia is usually not present in the fetal period because fetal bilirubin is transferred through the placenta and excreted by the mother. Neonatal hyperbilirubinemia arises from a transient deficiency of conjugation in the liver (exacerbated in premature neonates) and increased red cell turnover.[9]

HDFN is a complex disorder and disease severity seems to depend on many factors. These factors include: concentration, specificity, subclass, and glycosylation of antibodies; density, structure, and tissue distribution of antigens; efficiency of placental transport; functional maturity of the fetal spleen; macrophage Fc receptor function and the presence of HLA-related inhibitory antibodies.[10] In Kell HDFN for example, hyperbilirubinemia is usually not severe because anti-Kell antibodies cause suppression of erythropoiesis rather than hemolysis of erythrocytes.[11-13]

Prevalence

The exact prevalence of HDFN is difficult to determine because it depends on how HDFN is defined and the population in which it is measured. Reported prevalence of severe HDFN

varies from 3/100.000 to 80/100.000 pregnancies in developed countries.[14] In low income countries, prevalence of Rh negativity is lower than in North America and Europe. However, from data from three low income countries it is estimates that the prevalence of Rh HDFN varies from around 200/100.000 to around 340/100.000 births. In those countries there is a lack of Rh D prophylaxis leading to more neonates at risk.[15]

Prevention

Primary prevention of HDFN consists of extended matching of red blood cell (RBC) transfusions and the administration of Rh D immunoprophylaxis. In addition to ABO and Rh D matching of RBC transfusions, Dutch guidelines recommend matching for K, Rh c and Rh E for women below 45 years of age.[16] However, in many other countries this extensive matching is not routine practice. Rh D immunoprophylaxis has been licensed in Europe and North America since 1968. As a consequence, Rh D immunization rate decreased from 13.2% to 0.14% per Rh D positive pregnancy in Rh D negative mothers.[17] Nevertheless, maternal sensitization from fetomaternal hemorrhages will continue due to insufficient administration of Rh D immunoprophylaxis and the unavailability of immunoprophylaxis to prevent sensitization to non-Rh D antigens.

Antenatal management

Fetuses at risk are identified by maternal antibody screening and titration, determination of paternal antigen status, and determination of fetal antigen status.[18] The latter can be done by amniocentesis or noninvasively using free fetal DNA in maternal plasma. This noninvasive fetal blood group typing was first reported by Lo et al. in 1998.[19] Fetuses at risk were used to be monitored by amniotic fluid bilirubin measurements and cordocentesis to determine fetal anemia. However, currently noninvasive detection of fetal anemia by Doppler assessments of peak systolic velocity in the fetal middle cerebral artery is used predominantly.[20-22]

Treatment of fetal anemia by intrauterine intraperitoneal RBC transfusion was introduced in 1963.[23] In the 1980s intravascular transfusion via umbilical vessels became practice.[24,25] With improvement of this intravascular technique, nowadays overall perinatal survival rates in experienced centers vary between 89% and 92%.[26,27] Other proposed antenatal treatment options include maternal or fetal administration of intravenous immunoglobulin and maternal administration of phenobarbital.[29-32] However, further studies are needed to elucidate the role of those treatments.

Neonatal management

After birth, diagnosis of HDFN can be confirmed by blood group and Rh typing, measuring antibodies and bilirubin, and performing direct antibody (Coombs') test.[33]

Neonatal treatment of HDFN mainly consists of treatment of hyperbilirubinemia with phototherapy and exchange transfusion and correction of anemia. Severe (unconjugated) hyperbilirubinemia can lead to acute bilirubin encephalopathy and chronic bilirubin encephalopathy, also known as kernicterus.[34] Phototherapy, which was introduced in the 1970s, can prevent severe hyperbilirubinemia by photo-oxidation of bilirubin in the skin which converts it to a water-soluble substance. In the late 1940s treatment of neonatal hyperbilirubinemia with exchange transfusion (ET) was introduced.[35] ET not only removes bilirubin from the circulation to prevent kernicterus, but also maternal antibodies limiting further hemolysis. In addition, ET also corrects the associated anemia. ET is a high-risk invasive procedure and several alternative treatment options have been studied to reduce the need for ET, including intravenous immunoglobulin (IVIg), albumin, phenobarbarbital and metalloporphyrins. The use of the latter three options is not recommended since there is not sufficient evidence.[34] A Cochrane review on the use of IVIg in neonates with HDFN in 2002 concluded that IVIg was effective in reducing ET requirements. However, authors commented that evidence was limited due to small size and low quality of the studies and that routine use cannot be recommended.[34,36] Despite this advice, the guidelines of the American Academy of Pediatrics of 2004 advocate the routine use of IVIg in neonates with HDFN and consequently the use of it became widespread.[33,34]

Top-up transfusions, also known as simple or RBC transfusions, are used to treat anemia up to three months of age. In addition, several medicaments are used to stimulate erythropoiesis. These treatment options and the management of other hematological complications of HDFN are reviewed in this thesis.

Outline of the thesis

The Leiden University Medical Center (LUMC) is a tertiary center and the national referral center for intrauterine treatment of pregnancies complicated by red cell alloimmunization. Yearly around 35 neonates with HDFN due to red cell alloimmunization are admitted to the neonatal intensive care unit in the LUMC.

The aim of this thesis is to study the management and hematological outcome in neonates with HDFN.

The following chapters describe the objectives in more detail:

Chapter 2	Literature review on hematological morbidity and treatment options in HDFN due to red cell alloimmunization.
	Part 1: Exchange transfusions
Chapter 3	Study on the influence of a more restrictive ET guideline on the top-up transfusion requirements in neonates with Rh HDFN.
Chapter 4	Study on the morbidity associated with ET for red cell alloimmune hemolytic disease.
	Part 2: Intravenous immunoglobulin
Chapter 5	Randomized controlled trial on the use of IVIg in neonates with Rh HDFN (LIVIN study) investigating the effect of IVIg on the number of ETs.
Chapter 6	Cochrane systematic review on the use of IVIg in neonates with alloimmune hemolytic disease.
	Part 3: Alloimmunizations other than Rh D
Chapter 7	Study on the postnatal management and outcome in neonates with Kell hemolytic disease compared to Rh D hemolytic disease.
Chapter 8	Study on the postnatal management and outcome in neonates with Rh c hemolytic disease compared to Rh D hemolytic disease.

Part 4: Associated hematological morbidity

Chapter 9	Study on the incidence and severity of and risk factors for thrombocytopenia at birth in neonates with HDFN due to red cell alloimmunization.
Chapter 10	Study on the incidence and severity of and risk factors for cholestasis in neonates with HDFN due to red blood cell alloimmunization.
Chapter 11	Study on iron status during first three months of life in neonates with HDFN due to red cell alloimmunization.
Chapter 12	Summary and general discussion concerning the results of these studies.
Chapter 13	Future perspectives and proposals for future research.

References

1. Moise KJ, Jr. Management of rhesus alloimmunization in pregnancy. Obstet Gynecol. 2008;112:164-176.
2. Arnold DM, Smith JW, Kelton JG. Diagnosis and management of neonatal alloimmune thrombocytopenia. Transfus Med Rev. 2008;22:255-267.
3. Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? Transfusion. 2006;46:630-635.
4. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med. 2008;13:207-214.
5. Apkon M. Pathophysiology of hydrops fetalis. Semin Perinatol. 1995;19:437-446.
6. Pasman SA, van den Brink CP, Kamping MA, Adama van Scheltema PN, Oepkes D, Vandenbussche FP. Total blood volume is maintained in nonhydropic fetuses with severe hemolytic anemia. Fetal Diagn Ther. 2009;26:10-15.
7. Copel JA, Grannum PA, Green JJ, Belanger K, Hanna N, Jaffe CC, et al. Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion. Am J Obstet Gynecol. 1989;161:361-365.
8. al-Alaiyan S, al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med. 1999;27:112-115.
9. Maisels MJ, McDonagh AF. Phototherapy for neonatal jaundice. N Engl J Med. 2008;358:920-928.
10. Hadley AG. A comparison of in vitro tests for predicting the severity of haemolytic disease of the fetus and newborn. Vox Sang. 1998;74 Suppl 2:375-383.
11. Weiner CP, Widness JA. Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anemia. Am J Obstet Gynecol. 1996;174:547-551.
12. 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;171:247-252.
13. 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;338:798-803.
14. Koelewijn JM. Detection and prevention of pregnancy immunisation: The OPZI study University of Amsterdam, The Netherlands; 2009.[Thesis]
15. Zipursky A, Paul VK. The global burden of Rh disease. Arch Dis Child Fetal Neonatal Ed. 2011;96:F84-F85.
16. Quality institute of healthcare and evidence-based guideline development (CBO). [Guideline Blood Transfusion]. 2011 [Dutch]
17. Bowman J. Thirty-five years of Rh prophylaxis. Transfusion. 2003;43:1661-1666.
18. Geaghan SM. Diagnostic laboratory technologies for the fetus and neonate with isoimmunization. Semin Perinatol. 2011;35:148-154.
19. Lo YM, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med. 1998;339:1734-1738.
20. Moise KJ, Jr. The usefulness of middle cerebral artery Doppler assessment in the treatment of the fetus at risk for anemia. Am J Obstet Gynecol. 2008;198:161-164.

21. Zimmerman R, Carpenter RJ, Jr., Durig P, Mari G. Longitudinal measurement of peak systolic velocity in the fetal middle cerebral artery for monitoring pregnancies complicated by red cell alloimmunisation: a prospective multicentre trial with intention-to-treat. BJOG. 2002;109:746-752.
22. Oepkes D, Seaward PG, Vandenbussche FP, Windrim R, Kingdom J, Beyene J, et al. Doppler ultrasonography versus amniocentesis to predict fetal anemia. N Engl J Med. 2006;355:156-164.
23. Liley AW. Intrauterine transfusion of foetus in haemolytic disease. Br Med J. 1963;2:1107-1109.
24. Rodeck CH, Nicolaides KH, Warsof SL, Fysh WJ, Gamsu HR, Kemp JR. The management of severe rhesus isoimmunization by fetoscopic intravascular transfusions. Am J Obstet Gynecol. 1984;150:769-774.
25. Rodeck CH, Kemp JR, Holman CA, Whitmore DN, Karnicki J, Austin MA. Direct intravascular fetal blood transfusion by fetoscopy in severe Rhesus isoimmunisation. Lancet. 1981;1:625-627.
26. Tiblad E, Kublickas M, Ajne G, Bui TH, Ek S, Karlsson A, et al. Procedure-related complications and perinatal outcome after intrauterine transfusions in red cell alloimmunization in Stockholm. Fetal Diagn Ther. 2011;30:266-273.
27. Van Kamp IL, Klumper FJ, Oepkes D, Meerman RH, Scherjon SA, Vandenbussche FP, et al. Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red-cell alloimmunization. Am J Obstet Gynecol. 2005;192:171-177.
28. Van Kamp IL, Klumper FJ, Bakkum RS, Oepkes D, Meerman RH, Scherjon SA, et al. The severity of immune fetal hydrops is predictive of fetal outcome after intrauterine treatment. Am J Obstet Gynecol. 2001;185:668-673.
29. Ruma MS, Moise KJ, Jr., Kim E, Murtha AP, Prutsman WJ, Hassan SS, et al. Combined plasmapheresis and intravenous immune globulin for the treatment of severe maternal red cell alloimmunization. Am J Obstet Gynecol. 2007;196:138 e1-6.
30. Fox C, Martin W, Somerset DA, Thompson PJ, Kilby MD. Early intraperitoneal transfusion and adjuvant maternal immunoglobulin therapy in the treatment of severe red cell alloimmunization prior to fetal intravascular transfusion. Fetal Diagn Ther. 2008;23:159-163.
31. Connan K, Kornman L, Savoia H, Palma-Dias R, Rowlands S. IVIG - is it the answer? Maternal administration of immunoglobulin for severe fetal red blood cell alloimmunisation during pregnancy: a case series. Aust N Z J Obstet Gynaecol. 2009;49:612-618.
32. 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.
33. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114:297-316.
34. 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.
35. Wallerstein H. Treatment of Severe Erythroblastosis by Simultaneous Removal and Replacement of the Blood of the Newborn Infant. Science. 1946;103:583-584.
36. Alcock GS, Liley H. Immunoglobulin infusion for isoimmune haemolytic jaundice in neonates. Cochrane Database Syst Rev. 2002;CD003313.

Chapter 2

Hematological morbidity and management in neonates with hemolytic disease due to red cell alloimmunization

Mirjam E.A. Rath
Vivianne E.H.J. Smits-Wintjens
Frans J. Walther
Enrico Lopriore

Early Hum Dev 2011; 87(9):583-588

Introduction

Implementation of Rhesus (D) immunoprophylaxis in 1965 has led to a drastic decrease in incidence of hemolytic disease of the fetus and newborn (HDFN). Nevertheless, due to failure of pregnant women to obtain the prophylaxis and in a minor extent due to failure of the prophylaxis itself, anti-D is still the most commonly implicated antibody in HDFN. Other clinically relevant antibodies associated with severe HDFN are anti-Kell and anti-c.[1] Antenatal management of HDFN is based on the treatment of fetal anemia and prevention of fetal hydrops with intrauterine red cell transfusions (IUT). Since the introduction of IUT in 1963 by Sir William Liley, perinatal survival in HDFN has nowadays increased up to 90%.[2] Postnatal management in neonates with HDFN is based on the treatment of hyperbilirubinemia and prevention of kernicterus and includes mainly the use of phototherapy and exchange transfusions. In the last decade the use of intravenous immunoglobulin (IVIg) is increasingly being advocated. Postnatal treatment also consists of treating or preventing early and late anemia using top-up transfusions and supplements such as folic acid and iron. The additional use of erythropoietin treatment has also been studied in several small studies. In addition to anemia, other hematological complications such as thrombocytopenia, coagulation disturbances, leucopenia and iron overload have been reported. This review provides an overview of the hematological morbidity in HDFN due to red cell alloimmunization and summarizes the neonatal treatment options.

Anemia

Early versus late anemia: etiology

In red cell alloimmunization, anemia develops due to hemolysis of fetal red blood cells by maternal IgG antibodies directed against a paternal derived antigen which the mother is lacking. Maternal antibodies can persist for several months in the infants' circulation after birth causing ongoing hemolysis up to several months after birth. In neonates with HDFN early onset (within 7 days after birth) and late onset anemia are distinguished. Early onset anemia is caused by antibody dependent hemolysis of red blood cells. Late-onset anemia can be subdivided in "late hyporegenerative anemia" which is characterized by ineffective erythropoiesis and "late anemia of hemolytic disease" with an active bone marrow and appropriate levels of reticulocytes.[3] It should be noted that these types of anemia can occur simultaneously. More information on the types of anemia in HDFN is presented in table 1.

Table 1. Characteristics of early versus late onset anemia in red cell alloimmunization

		Late onset anemia	
	Early onset anemia	Late hyporegenerative anemia	Late anemia of hemolytic disease
Onset of anemia	Within 7 days of age	After ≥2 weeks of age	
Etiologic mechanisms	Antibody-dependent hemolysis of RBCs	1) antibody-dependent destruction of RBC-precursors, reticulocytes and erythrocytes; 2) marrow suppression by IUTs and postnatal RBC-transfusions; 3) erythropoietin deficiency; 4) shortened half-life of transfused RBCs; 5) expanding intravascular volume of growing infant	1) antibody-dependent hemolysis of RBCs; 2) shortened half-life of transfused RBCs; 3) natural decline of Hb levels; 4) expanding intravascular volume of growing infant
Bilirubin level	Elevated	Normal	Usually elevated
Reticulocyte count	Normal or high	Low or absent	Normal or high

RBC = red blood cell; IUT = intrauterine red cell transfusion; Hb = hemoglobin

Late anemia is a common problem in infants with HDFN. Al-Alaiyan et al. found an incidence of late anemia (hemoglobin <8 g/dL = 5 mmol/L) in 83% (30/36) in term and late-preterm infants with HDFN.[3] Because of the risk for prolonged anemia, a full work-up including invasive diagnostic tests (such as bone marrow aspirations) to exclude other causes of anemia is generally not necessary and should only be considered in select cases with an abnormal or persistent course.

Although late anemia was already described before the use of IUT, treatment with IUT seems to increase and prolong the risk of anemia, probably by reducing erythropoiesis.[4]

A high persistent antibody titer in Rhesus D hemolytic disease can also contribute to prolonged anemia.[5] In contrast, in Kell HDFN antibody titer does not correspond well with the severity of anemia. Anemia in Kell HDFN seems to result from reduced erythropoiesis caused by destruction of progenitor red blood cells rather than hemolysis of erythrocytes.[6]

Although fetal anemia in Kell HDFN is often more severe necessitating more often IUT treatment compared to Rhesus D HDFN, in the neonatal period no significant differences

Table 2. Information on studies (published in English) on rhEPO administration to neonates with HDFN due to red cell alloimmunization

First author/year of publication	cases	GA (weeks)	Type of antibody	Number of IUTs/top-ups/ETs before rhEPO
Ohls/ 1992[13]	2	34.5; 32	Rhesus D	both 4 / case 1:2, case 2:1/ 0
Scaradavou/ 1993[15]	4	35.5-37	Rhesus D	2-5 / 0-3 / 0
Zuppa/ 1999[17]	6	28-38	Rhesus	1-9 / 0 / 0-3
Ovaly/ 1996 (RPCT)[14]	10/10	≥35	Rhesus	rhEPO group: 2.4 * / 2 cases** / 1 case**; control group: 2.0* / 3 cases** / 1case**
Wacker/ 2001[16]	1	38	Rhesus E	0 / 0 / 0
Dhodapkar/ 2001[10]	1	35	Kell	0 / 0 / 0
Nicaise/ 2002[12]	2	35	Rhesus; Rhesus D	both 4 IUET/ both 2 / 0
Pessler/ 2002[20]	1	35	Rhesus D	4 / 2 / 0
Ovaly/ 2003 (Letter)[19]	103	NS	NS	NS
Manoura/ 2007[11]	1	36	Kell (Ku)	Regular IUTs / 0 / 0
Zuppa/ 2010[18]	25	33 ± 3*	Rhesus	In 14 of 25 IUT(s): 3 ± 2* / 4 cases of ET for anemia / 20 (incl.4 for anemia) cases ≥1 ET
Masumoto/ 2010[44]	1	37	Jra	0 / 0 / 0

rhEPO = recombinant human erythropoietin; HDFN = hemolytic disease of the fetus and newborn; GA = gestational age; top-ups = top-up transfusions; ET = exchange transfusion; NS = not specified; IVIg = intravenous immunoglobulin; RPCT = randomized placebo controlled trial; IUET = intrauterine exchange transfusion; * mean (± standard deviation); ** number of transfusions not specified; *** 200 U/kg/d (3×/wk) for 4 weeks, then 150 U/kg/d (3×/wk) for 5 weeks

Dose of rhEPO	Start rhEPO (d after birth)	Duration of rhEPO	Side effects	Supplements	Top-ups after start rhEPO
200 U/kg/d	85; 82	9 and 10 d	neutropenia in case 1	NS	0
200 U/kg 3×/wk	7-52	Stopped in case of reticulocytosis + Hb increase of ≥1 g/dL	none	iron, folic acid	0,1,1,1
200 U/kg/d	14-17	3 weeks	none	iron, folic acid, vitamin E	0,2,1,0,2,3
200 U/kg 3×/wk	14	6 weeks	none	iron, folic acid, IVIg	rhEPO group: 1.8* control group: 4.2*
870 U/kg/d	13	1 month	mild splenomeg-aly, discomfort injection sites	iron, folic acid	0
150-200 U/kg 3×/wk***	14	9 weeks	none	iron	0
250 U/kg 3×/wk	73; 71	6 and 3.4 weeks	NS	case 1: iron, folic acid, IVIg case 2: IVIg	0
200 U/kg 3×/wk	8	5 weeks	(iron overload)	iron	4
200 U/kg 3×/wk	14	6 weeks	NS	IVIg	1.5* (55% none)
300 U/kg 3×/wk	15	8 weeks	NS	iron	0
400 U/kg/d (except first 6 neonates: 200 U/kg/d)	11 ± 4*	26 ± 14* d	none	iron, folic acid and vitamin E	only in IUT treated neonates: 1.8 ± 0.7*
NS (7×rhEPO from d 20-60)	20	6 weeks	NS	iron	0

in top-up transfusion requirements are seen.[6] Due to the low incidence of antibodies other than anti-Rhesus D and anti-Kell, studies in non-Rhesus D and non-Kell HDFN with sufficient sample size to make reliable conclusions on the risk of (late) anemia are lacking.

Top-up transfusions

Top-up transfusions (or simple or erythrocyte transfusions) to treat postpartum anemia can be necessary up to the third month of life.[7] Approximately 80% of infants with HDFN treated with IUT require at least one top-up transfusion for late anemia (hemoglobin <5 mmol/L = <8 g/dL) during the first 3 months.[3,7,8] In infants with HDFN who did not receive IUTs, around 65% require at least one top-up transfusion.[3] Median number of top-up transfusions required in Rhesus D HDFN and Kell HDFN are two (IQR 0-2) and one transfusion (IQR 0-2), respectively. However, in some cases up to six top-up transfusions are necessary to treat late anemia.[6]

International guidelines for top-up transfusions including transfusion triggers during the first months of life are not available. Various protocols based on clinical condition, respiratory support, oxygen supplementation, postnatal age, hemoglobin and hematocrit levels and weight gain are used.[9] In our centre top-up transfusion triggers in term neonates with HDFN include: hemoglobin levels <8.0 g/dL (5.0 mmol/L) or <9.6 g/dL (6.0 mmol/L) when clinical symptoms of anemia are present like need of extra oxygen, poor feeding, tachycardia and/or tachypnea.

The vast majority of top-up transfusions given to neonates with HDFN consist of 10-20 ml/kg (weight on day of transfusion) ABO/Rhesus type-specific and antigen-negative (for maternal antibodies) red blood cells. Due to an incompletely developed immune system in (premature) neonates, irradiation of cellular blood components and cytomegalovirus and parvo-B19 virus risk reduction are important safety measures.[9] Furthermore, the use of satellite packs (one unit of donor blood divided in smaller aliquots) is valuable for reducing donor exposure.

Erythropoietin

Some authors advocate the use of Erythropoietin (EPO) to prevent late anemia in neonates with HDFN.[10-19] EPO is a glycoprotein hormone responsible for fetal and neonatal erythropoiesis and increases on hypoxic stimuli. The normal EPO-level in the non-anemic full-term infants at 7-50 days of age was estimated to be 30 mU/ml.[3] In the fetal period EPO is produced in the liver and does not cross the placenta and after birth production shifts to the kidneys. In HDFN EPO-production can be insufficient for the degree of anemia.[18]

Since 1992 several reports on the use of recombinant human erythropoietin (rhEPO) in

neonates with HDFN have been published (table 2). Most reports showed beneficial effects of rhEPO treatment without any side effects except for two cases. However, reported studies were small case series or case reports and only one small randomized controlled trial has been performed. In addition, gestational ages in these studies varied between 28 weeks and 40 weeks and protocols varied greatly in the timing, duration and dosages of rhEPO and top-up transfusion triggers. In the study of Zuppa et al. only IUT treated neonates needed top-up transfusions after rhEPO treatment.[18] Theoretically, this could be due to an insufficient dose of rhEPO to compensate for the reduced endogenous EPO-production caused by transfused adult hemoglobin (HbA) which provides more oxygen to tissues.[18] In addition, Pessler et al. suggested that rhEPO treatment might be ineffective when antibody titers are high.[20]
Unfortunately effective rhEPO treatment can only be administered subcutaneously and intravenously.

Long-acting erythropoietin
In three small pharmacokinetic and pharmacodynamic studies in premature infants long-acting erythropoietin (darbepoetin) which can be applied once weekly was considered as a reasonable alternative to rhEPO with a shorter half-life, but larger randomized trials are needed before darbepoetin can be recommended for routine clinical use in neonates.[21-23]

Supplements to support erythropoiesis
For effective erythropoiesis sufficient amounts of iron, folic acid and vitamin B12 are required. Folate and vitamin B12 are essential for proliferation of erythroblasts during their differentiation and iron is required for hemoglobin synthesis by erythroblasts. Deficiency of one these nutrients results in ineffective erythropoiesis.[24]

Folic acid
Folic acid is frequently supplemented although limited data are available on its beneficial effect on erythropoiesis in infants with HDFN. Gandy and Jacobson demonstrated prospectively that oral folic acid supplementation (2.5-5 mg/d) has a beneficial effect on growth and serum folate levels, but not on hemoglobin levels in erythroblastotic infants.[25] Strelling et al. did found a hematological response in two severely anemic infants after daily administration of intramuscular folic acid.[26]

Although adverse effects of severe maternal folate deficiency on the motor and mental development of infants have been reported, the role of low serum folate levels during the first year of life on neurodevelopmental outcome is unclear.

Folic acid dosages reported in the literature vary from 0.025 to 5 mg/d and side effects (such as rash, fever) are uncommon.
Even though high-leveled evidence is lacking, in our centre we routinely supplement 0.05 mg/d orally to infants with HDFN during the first three months of life.

Iron

Iron supplementation is also used to support erythropoiesis in anemic neonates with HDFN. However, neonates with HDFN usually do not lack iron. On the contrary, in Rhesus HDFN ferritin levels (which reflects total body iron stores) in fetal and cord blood are highly elevated.[27,28] Moreover, in HDFN blood transfusions are frequently necessary and with each top-up transfusion, iron is transfused as well. Multiple intrauterine and/or postnatal blood transfusions can lead to iron overload that exceeds transferrin binding capacity. Non-tranferrin bound iron can lead to the formation of hydroxyl radicals which can damage lipids, proteins, sugars and DNA.[27] Additionally, iron overload can cause damage to the liver, heart and endocrine organs and also can alter immune response and increase susceptibility to infection.[29] Nevertheless, iron is crucial for early brain growth and function and iron deficiency as well as iron overload have been associated with neurodevelopmental impairment.[29]
The incidence and morbidity of iron overload and long-term neurodevelopmental outcome in HDFN is unclear. Therefore, iron supplementation should be withheld, especially in transfused infants with HDFN, unless ferritin levels are in the low/normal ranges. More research is necessary to define indications for chelation therapy, which occasionally is used to treat iron overload in HDFN.[30]

Vitamin E

Occasionally vitamin E is supplemented to neonates with HDFN. Vitamin E is an antioxidant which reduces oxidative stress to the red blood cell membrane. Therefore vitamin E deficiency can generate a shorter life span of red blood cells. Serum vitamin E (tocopherol) concentrations in newborns are abnormally low compared to adult concentrations and administration of vitamin E to newborns protects against hemolysis by hydrogen peroxide in vitro.[31] In contrast, addition of a large amount of vitamin E analogue did not inhibit the peroxidation process in three neonates with rhesus hemolytic disease.[27] These limited data are inconclusive to routinely advise on the use of vitamin E in HDFN.

Exchange transfusion

Exchange transfusions (ET) in HDFN due to red cell alloimmunization are performed to wash out the increased bilirubin and maternal red blood cell antibodies. In addition to removal of antibodies, the neonatal red blood cells are replaced by immunologically compatible cells which survive in the blood stream, hence reducing the hemolytic process and the risk of neonatal anemia.[8] Another favorable effect of ET is a fall in ferritin and iron plasma concentrations.

However, ET are also known to be associated with adverse effects such as catheter related infections, thrombosis and hemorrhage.[7] In 2004 more restrictive ET guidelines were published by the American Academy of Pediatrics (AAP) and led consequently to a reduction in the use of ET.[8] This reduction in ET has led to an increased need of top-up transfusions caused by the ongoing hemolysis and remaining antibodies.[8]

Treatment with intravenous immunoglobulin (IVIg)

Although the mechanism of intravenous immunoglobulin in HDFN is incompletely understood, IVIg is applied to reduce the need for ET in case of failure of phototherapy to treat hyperbilirubinemia. In small randomized controlled trials IVIg reduced the need for ET and duration of phototherapy in neonates with Rhesus HDFN, but the need for late top-up transfusions was increased. However, these studies were restricted by several important methodological limitations. In 2002, a Cochrane review was published and concluded that more well-designed trials are needed before routine use of IVIg can be recommended for treatment of HDFN.[32] In 2006, Nasseri et al. described a reduction in the number of ETs and duration of phototherapy after a 3-dose treatment with IVIg in neonates with Rhesus and ABO hemolytic disease.[33] Although it seems a randomized controlled trial (RCT), they did not describe the randomization process and whether the study was blinded, and moreover the number of neonates with Rhesus HDFN was small. In addition, the 100% need for ET in the control group of Rhesus HDFN indicates a suboptimal postnatal treatment with phototherapy.[33]

In 2011, Elalfy et al. performed an RCT on the use of early IVIg in 90 term (>38 weeks' gestation) neonates with Rhesus HDFN not formerly treated with IUT. They concluded that IVIg administration at 12 h after birth was effective in reducing duration of phototherapy and the need for ET.[34] However, in this unblinded study the randomization process was also not specified and parents were even allowed to choose whether their child would receive IVIg and in which dose after randomization was performed. In contrast, our study group recently concluded a double-blind randomized placebo controlled trial on the prophylactic use of IVIg in Rhesus HDFN and demonstrated that IVIg does not reduce the need for ET

nor the rates of other adverse neonatal outcomes.[35] Recently, a research group from Brazil finalized a similar RCT on IVIg for neonates with Rhesus hemolytic disease and also found no difference between both groups on the rate of ET.[36] A new meta-analysis of all recently performed RCTs is required to determine the efficacy and safety of IVIg in HDFN due to red cell alloimmunization. In view of the absence of beneficial effects and because of potential (but rare) adverse effects associated with the use of IVIg such as transfusion-transmitted diseases, anaphylaxis, hypersensitivity, thrombosis, pulmonary emboli, and renal failure, we do not recommend the routine use of IVIg.[35]

Thrombocytopenia

Incidence of thrombocytopenia in HDFN

The association between thrombocytopenia and red cell alloimmunization was first described more than 50 years ago.[37] However, since then, the prevalence, severity and risks of thrombocytopenia in the neonatal period have not been investigated. In a small study in Rhesus HDFN by Koenig et al., all of the severely diseased (hydropic, n = 5) and moderate severely diseased (non-hydropic but requiring ET, n = 6) infants but none of the mildly diseased (not treated with ET, n = 9) infants had thrombocytopenia.[38]

Two recent studies demonstrated that thrombocytopenia (platelet count $<150 \times 10^9$/L) is present in the fetal period in 26% and 10% of neonates treated with IUT for Rhesus D and Kell HDFN, respectively.[39,40] In Rhesus D but not in Kell HDFN, hydrops was associated with fetal thrombocytopenia. In surviving fetuses with Rhesus HDFN, significantly more fetuses with severe thrombocytopenia (platelet count $<50 \times 10^9$/L) had an intracranial bleeding on cranial ultrasound in the first week of life compared to fetuses without severe thrombocytopenia.[39]

To elucidate the incidence, etiology and complications of neonatal thrombocytopenia in red cell alloimmunization further research is necessary. We recently performed a retrospective observational study on the incidence and severity of thrombocytopenia in red cell alloimmunization and results will be awaited soon.

Etiology of thrombocytopenia in HDFN

Based on hematopoietic progenitor cell studies, it appears that because of increased erythropoiesis in fetuses with severe Rhesus HDFN neutrophil and platelet production can be suppressed.[38] In addition to a decreased production, increased destruction or consumption and dilution may also play a role. Thrombocytopenia is a known complication of ET caused by platelet-poor blood and/or catheter-related thrombi.[41] Bilirubin toxicity to

platelets leading to changes in platelet morphology and possibly to platelet destruction is described, but seems irrelevant since it has only been seen in very high levels of bilirubin.[38]

Coagulation

Thirty years ago prospective studies on coagulation status showed a coagulation failure in neonates with severe HDFN. A platelet count of less than 150 × 10^9/L and severe deficiency of multiple coagulation factors were described.[42] The severely diseased neonates with coagulation failure died of bleeding complications in longs or brain. The authors presumed that the very low vitamin-K dependent factor levels in the cord blood were the result of liver damage in utero. Vitamin-K prophylaxis was administered at birth and in some cases in subsequent days. Use of ET seemed to correct the factor deficiency temporarily and platelet count decreased in neonates with platelets >50 × 10^9/L and increased in neonates with platelet counts <50 × 10^9/L. Coagulation tests at birth (including prothrombin and thrombin time) appeared to identify the neonates at risk of bleeding complications.[42] Since intrauterine treatment has improved greatly over the years, severe hemolytic disease with hydrops is rare nowadays. However, in case of severe hydropic disease there is still a risk for serious coagulation failure with subsequent risk for bleeding complications.

Leucopenia

Very limited data are available on leucopenia in neonates with HDFN due to red cell alloimmunization. Koenig et al. reported that of 20 patients with Rhesus HDFN, all 5 neonates with severe Rhesus HDFN, two of six with moderately severe disease, and two of nine with mild disease had neutropenia.[38] It appears that the incidence of neutropenia increases if Rhesus HDFN is more severe. Segal et al. described two neonates with Rhesus incompatibility, hydrops fetalis, and neutropenia who were successfully treated with recombinant human granulocyte colony-stimulating factor (rhG-CSF) without any side effects.[43] These limited data are insufficient to be conclusive on the incidence and morbidity of neutropenia and further investigations are needed.

Key guidelines

- In the majority of neonates with HDFN late anemia is present necessitating top-up transfusions up to three months after birth.
- Iron supplementation should be withheld, especially in transfused infants with HDFN, until ferritin levels are normalized.
- IVIg does not reduce the need for ET in neonates with Rhesus HDFN and the routine use of IVIg should therefore be discouraged.

Research directions

- More studies are needed to determine the incidence and risk factors of iron overload in infants with HDFN treated with and without IUT.
- To elucidate the prevalence and morbidity of neonatal thrombocytopenia and the efficacy of prophylactic platelet transfusions in HDFN, further research is required.
- Larger well-designed trials are needed to recommend on the use of rhEPO therapy in neonates with HDFN.
- Studies on the use of folic acid in neonates with HDFN are needed to determine if and in which dosage this therapy could be beneficial.

References

1. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med 2008;13:207-14.
2. Van Kamp IL, Klumper FJ, Oepkes D, Meerman RH, Scherjon SA, Vandenbussche FP et al. Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red-cell alloimmunization. Am J Obstet Gynecol 2005;192:171-7.
3. al-Alaiyan S, al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med 1999;27:112-5.
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. Dorn I, Schlenke P, Hartel C. Prolonged anemia in an intrauterine-transfused neonate with Rh-hemolytic disease: no evidence for anti-D-related suppression of erythropoiesis in vitro. Transfusion 2010;50:1064-70.
6. Rath ME, Smits-Wintjens VE, Lindenburg IT, Brand A, Van Kamp IL, Oepkes D et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100:312-6.
7. 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;13:265-71.
8. Rath ME, Smits-Wintjens VE, Lindenburg I, Brand A, Oepkes D, Walther FJ et al. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010.
9. New HV, Stanworth SJ, Engelfriet CP, Reesink HW, McQuilten ZK, Savoia HF et al. Neonatal transfusions. Vox Sang 2009;96:62-85.
10. Dhodapkar KM, Blei F. Treatment of hemolytic disease of the newborn caused by anti-Kell antibody with recombinant erythropoietin. J Pediatr Hematol Oncol 2001;23:69-70.
11. Manoura A, Korakaki E, Hatzidaki E, Saitakis E, Maraka S, Papamastoraki I et al. Use of recombinant erythropoietin for the management of severe hemolytic disease of the newborn of a K0 phenotype mother. Pediatr Hematol Oncol 2007;24:69-73.
12. 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;17:22-4.
13. Ohls RK, Wirkus PE, Christensen RD. Recombinant erythropoietin as treatment for the late hyporegenerative anemia of Rh hemolytic disease. Pediatrics 1992;90:678-80.
14. 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;39:831-4.
15. 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;123:279-84.
16. Wacker P, Ozsahin H, Stelling MJ, Humbert J. Successful treatment of neonatal rhesus hemolytic anemia with high doses of recombinant human erythropoietin. Pediatr Hematol Oncol 2001;18:279-82.

17. Zuppa AA, Maragliano G, Scapillati ME, Florio MG, Girlando P, Noia G et al. Recombinant erythropoietin in the prevention of late anaemia in intrauterine transfused neonates with Rh-haemolytic disease. Fetal Diagn Ther 1999;14:270-4.
18. Zuppa AA, Alighieri G, Calabrese V, Visintini F, Cota F, Carducci C 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.
19. Ovaly F. Late anaemia in Rh haemolytic disease. Arch Dis Child Fetal Neonatal Ed 2003;88:F444.
20. Pessler F, Hart D. Hyporegenerative anemia associated with Rh hemolytic disease: treatment failure of recombinant erythropoietin. J Pediatr Hematol Oncol 2002;24:689-93.
21. Warwood TL, Ohls RK, Wiedmeier SE, Lambert DK, Jones C, Scoffield SH et al. Single-dose darbepoetin administration to anemic preterm neonates. J Perinatol 2005;25:725-30.
22. Warwood TL, Ohls RK, Lambert DK, Leve EA, Veng-Pedersen P, Christensen RD. Urinary excretion of darbepoetin after intravenous vs subcutaneous administration to preterm neonates. J Perinatol 2006;26:636-9.
23. Warwood TL, Ohls RK, Lambert DK, Jones C, Scoffield SH, Gupta N et al. Intravenous administration of darbepoetin to NICU patients. J Perinatol 2006;26:296-300.
24. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004;24:105-31.
25. Gandy G, Jacobson W. Influence of folic acid on birthweight and growth of the erythroblastotic infant. III. Effect of folic acid supplementation. Arch Dis Child 1977;52:16-21.
26. Strelling MK, Blackledge GD, Goodall HB, Walker CH. Megaloblastic anaemia and whole-blood folate levels in premature infants. Lancet 1966;1:898-900.
27. 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;335:933-6.
28. Nasrat HA, Nicolini U, Nicolaidis P, Letsky EA, Gau G, Rodeck CH. The effect of intrauterine intravascular blood transfusion on iron metabolism in fetuses with Rh alloimmunization. Obstet Gynecol 1991;77:558-62.
29. 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.
30. Yilmaz S, Duman N, Ozer E, Kavas N, Oren H, Demircioglu F et al. A case of rhesus hemolytic disease with hemophagocytosis and severe iron overload due to multiple transfusions. J Pediatr Hematol Oncol 2006;28:290-2.
31. Villalaz RA, Toner N, Chiswick ML. Dietary vitamin E and polyunsaturated fatty acid (PUFA) in newborn babies with physiological jaundice. Early Hum Dev 1981;5:145-50.
32. Alcock GS, Liley H. Immunoglobulin infusion for isoimmune haemolytic jaundice in neonates. Cochrane Database Syst Rev 2002;CD003313.
33. Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. Saudi Med J 2006;27:1827-30.
34. Elalfy MS, Elbarbary NS, Abaza HW. Early intravenous immunoglobin (two-dose regimen) in the management of severe Rh hemolytic disease of newborn-a prospective randomized controlled trial. Eur J Pediatr 2011;170:461-7.

35. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, Te Pas AB, Kramer CM et al. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011;127:680-6.
36. Santos MC, Sa CA, Gomes SC, Camacho LA, Moreira ME. High-dose intravenous immunoglobulin therapy for hyperbilirubinemia due Rh hemolytic disease: a randomized clinical trial. Pediatric Academic Societies-annual meeting-Vancouver 2010 (E-PAS2010:2851.333), 143. 2010.
37. CLINICAL pathological conference. J Pediatr 1957;50:490-8.
38. Koenig JM, Christensen RD. Neutropenia and thrombocytopenia in infants with Rh hemolytic disease. J Pediatr 1989;114:625-31.
39. 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;199:387-4.
40. Van den Akker ES, Klumper FJ, Brand A, Kanhai HH, Oepkes D. Kell alloimmunization in pregnancy: associated with fetal thrombocytopenia? Vox Sang 2008;95:66-9.
41. Chadd MA, Gray OP, Hole DJ. Blood coagulation studies during exchange transfusion. J Obstet Gynaecol Br Commonw 1972;79:373-6.
42. Hey E, Jones P. Coagulation failure in babies with rhesus isoimmunization. Br J Haematol 1979;42:441-54.
43. Segal N, Leibovitz E, Juster-Reicher A, Even-Tov S, Mogilner B, Barak Y. Neutropenia complicating Rh-hydrops fetalis: the effect of treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF). Pediatr Hematol Oncol 1998;15:193-7.
44. Masumoto A, Masuyama H, Sumida Y, Segawa T, Hiramatsu Y. Successful management of anti-Jra alloimmunization in pregnancy: a case report. Gynecol Obstet Invest 2010;69:81-3.

Part 1

Exchange transfusions

Chapter 3

Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions

Mirjam E.A. Rath
Vivianne E.H.J. Smits-Wintjens
Irene T.M. Lindenburg
Anneke Brand
Frans J. Walther
Enrico Lopriore

Vox Sang 2010; 99(1):65-70

Abstract

Objective

To study the effect of a restrictive guideline for exchange transfusion (ET) on the number of top-up transfusions in neonates with Rhesus hemolytic disease.

Study design

Retrospective study of all (near)-term neonates with Rhesus hemolytic disease admitted to our center between 2000 and 2008. In December 2005, policy changed from using liberal ET criteria to more restrictive ET criteria. We recorded the number of ETs and the number of top-up transfusions in the group of neonates before (group I, n = 156) and after (group II, n = 27) the guideline change.

Results

The percentage of neonates requiring an ET decreased from 66% (103/156) in group I to 26% (7/27) in group II (p = <0.01). The percentage of neonates receiving a top-up transfusion increased from 68% (105/154) in group I to 81% (22/27) in group II (p = 0.25). The median number of top-up transfusions increased from 1 (interquartile range 0-2) in group I to 2 (interquartile range 1-3) in group II (p = 0.01).

Conclusion

In this study, restrictive ET criteria in neonates with Rhesus hemolytic disease lead to a reduction of the rate of ET but an increase in the number of top-up transfusions for neonatal anemia.

Introduction

The mainstay of antenatal treatment for hemolytic disease of the fetus and newborn due to Rhesus alloimmunization (HDFN) is intrauterine transfusions (IUT) to treat severe fetal anemia. The mainstay of postnatal treatment for hemolytic disease of the newborn (HDN) secondary to Rhesus alloimmunization is (1) intensive phototherapy and exchange transfusion (ET) to treat hyperbilirubinemia and prevent kernicterus, and (2) top-up transfusions to treat neonatal anemia. Neonatal anemia secondary to Rhesus alloimmunization can be divided into two types: "hyporegenerative anemia" characterized by depressed erythropoiesis and "late anemia of hemolytic disease" caused by persisting hemolysis by remaining antibodies.[1] Both causes of anemia contribute to the necessity of top-up transfusions during the first months of life. The percentage of infants with HDN secondary to Rhesus alloimmunization requiring top-up transfusions for neonatal anemia varies from 27 to 83%.[2,3]

Several risk factors for neonatal anemia secondary to Rhesus alloimmunization have been reported, including IUT (due to suppression of fetal erythropoiesis)[2] and severity of HDN.[1,4,5] Use of ET during the neonatal period has been reported to protect against neonatal anemia.[3] In addition to removing excess bilirubin, ET also removes antibody-coated erythrocytes and maternal antibodies, hence reducing the risk for continuing hemolysis and neonatal anemia.[3,6] However, the protective role of ET for neonatal anemia has only been demonstrated in one small study.[3]

In December 2005, we implemented a new guideline for the management of neonatal hyperbilirubinemia and HDN secondary to Rhesus alloimmunization based on the guidelines from the American Academy of Pediatrics (AAP).[7] The new guideline for ET was more restrictive than the previous ones and led to a more than 50% decrease in the rate of ET. Restrictive ET criteria may hypothetically lead to an increase in top-up transfusions ascribed to ongoing hemolysis due to antibody coated cells not being removed from the circulation. Whether the reduction in ET indeed resulted in an increased rate of top-up transfusions was not clear.

The aim of this study was to evaluate the effect of this new guideline on the number of top-up transfusions and determine if neonates with hemolytic disease of the newborn secondary to Rhesus alloimmunization treated with ET are less likely to develop neonatal anemia.

Patients and Methods

All neonates with a gestational age ≥35 weeks with HDN secondary to Rhesus D, C, c or E antibodies admitted between January 2000 and November 2008 to the neonatal division of the Leiden University Medical Center (LUMC) were included in this retrospective observational study. LUMC is the national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. Neonates with Kell, Jka or Cw red cell alloimmunization, and neonates receiving transfusions for unrelated pathology were excluded from this study. We also excluded neonates participating in an ongoing randomized double-blind placebo-controlled trial for the use of immunoglobulin in RHD, which started in August 2006 at our institution (the LIVIN-study: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=832)

In December 2005, we implemented a new guideline for the management of neonatal hyperbilirubinemia and HDN secondary to Rhesus alloimmunization based on the guidelines from the American Academy of Pediatrics (AAP).[7] The differences in phototherapy thresholds before and after introduction of the new guideline are shown in figure 1. The guideline for phototherapy administration at our neonatal division has previously been described.[2] The bilirubin thresholds for ET in the new guideline were higher than in the previous one. The differences in ET thresholds of total serum bilirubin before and after guideline change are shown in figure 2. The criteria for ET before December 2005 included a total serum bilirubin level at birth >3.5 mg/dL (measured in neonatal blood at birth or in a few cases in umbilical cord blood), (early criterion) and/or a total serum bilirubin level above ET thresholds (rise of bilirubin value >0.5 mg/dL/hr despite intensive phototherapy).[2] In neonates not treated with IUT, a hemoglobin level at birth of <12.9 g/dL was also considered as an early criterion for ET.[2] Bilirubin levels were measured in all neonates every 2 to 3 hours during the first few days after birth.

Early criteria for ET were abandoned after the guideline change. The criteria for ET after December 2005 were: (1) total serum bilirubin above (new) ET thresholds[7] (fig 2) and/or (2) rise of bilirubin >0.5 mg/dL/hr despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level. ET was performed with double-volume transfusion (160 mL/kg) using irradiated and leukocyte-depleted compatible erythrocytes.

After initial discharge from the LUMC, top-up transfusions were performed when hemoglobin levels were <8.0 g/dL or <9.6 g/dL if clinical symptoms of neonatal anemia (lethargy, feeding problems, need for oxygen or failure to thrive) were present. The volume used for top-up transfusions during the study period was 15 ml/kg bodyweight. The volume

Figure 1. Phototherapy thresholds before and after guideline change in neonates with Rh hemolytic disease

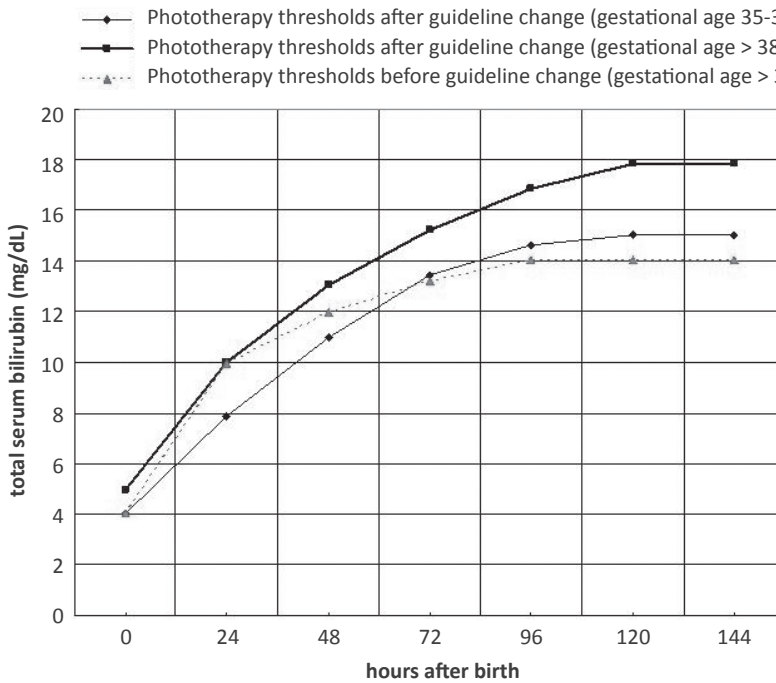
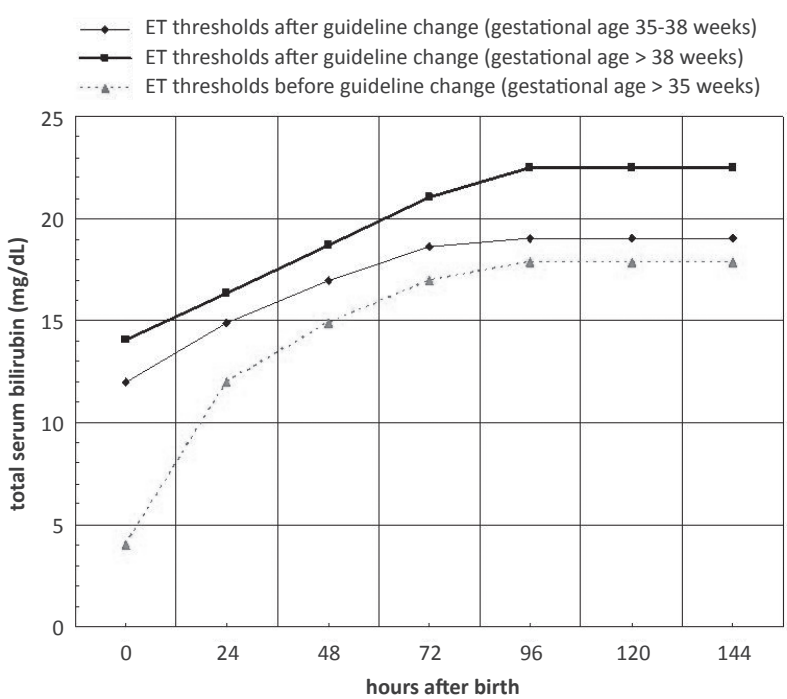


Figure 2. Exchange transfusion thresholds before and after guideline change in neonates with Rh hemolytic disease



and criteria for top-up transfusion were the same in both groups. Neonatal anemia, often referred to as "late anemia" in previous studies[3,5,8,9] was defined as a hemoglobin level below thresholds requiring a top-up transfusion during the first three months of life. Folic acid 50 mcg/day was administered orally during the first three months of life to all neonates with RHD. According to our management guidelines, neonates with RHD were not treated with erythropoietin.

We recorded the following obstetric and neonatal data: fetal hemoglobin at first IUT and number of IUTs, gestational age at birth, birth weight, hemoglobin level and reticulocyte count at birth, bilirubin level at birth, maximum bilirubin level during admission, duration of phototherapy (days), number of ETs required, number of top-up red blood cell transfusions received during the first 3 months of life and hemoglobin levels prior to the top-up transfusion. Obstetric data were gathered using a dedicated database for Rhesus complicated pregnancies in which data are prospectively collected. Neonatal data were collected using medical files. Data on top-up transfusions and hemoglobin levels prior to the top-up transfusion in infants who received follow-up outside the LUMC were gathered through correspondence with the local pediatrician or blood transfusion department.

Primary outcome was the number of top-up transfusions in the group of neonates admitted before (group I) and after (group II) the new guideline implementation in December 2005. Data are reported as means and standard deviations (SD) or as medians and interquartile range (IQR), as appropriate. Statistical analysis was performed using Student-t-test and Mann-Whitney test for continuous variables. Chi square and Fisher's exact test were used for categorical variables, as appropriate. To assess the relationship between the number of ETs and the number of top-up transfusions, Spearman correlations were calculated. A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 16.0 (SPSS Inc, Chicago, IL).

Results

During the study period 270 infants with hemolytic disease born at ≥35 weeks' gestation were admitted to our neonatal division. Fifty neonates were excluded because of participation in a randomized double-blind placebo-controlled trial for the use of intravenous immunoglobulin. Thirty-six neonates were excluded due to hemolytic disease caused by Kell (n = 34), Jka (n = 1) or Cw (n = 1). One neonate requiring major cardio-thoracic surgery for congenital heart disease was also excluded. A total of 183 patients were included in this study, of whom 156 in the group before (group I) and 27 in the group after (group II) the implementation of the new guideline in December 2005. Baseline

characteristics in both groups are summarized in Table 1.

Table 1. Baseline characteristics in neonates with Rhesus hemolytic disease in group I and group II

	Group I (n = 156)	**Group II** (n = 27)	**p-value**
Neonates treated with IUT, n (%)	99 (63)	15 (56)	0.52
Number of IUTs per neonate[a]	2 (0 - 3)	1 (0 - 2)	0.29
Gestational age at first IUT, weeks[a]	30 (24 – 33)	31 (23 - 32)	0.70
Hemoglobin level at first IUT, g/dL[a]	6.6 (5.1 – 7.7)	5.8 (5.0 – 7.4)	0.70
Gestational age at birth, weeks[a]	37 (36 - 37)	37 (36 - 37)	0.89
Birth weight, grams[b]	3020 ± 445	2940 ± 396	0.40
Male, n (%)	87 (56)	21 (78)	0.04
Neonates with Rhesus D, n (%)	140 (90)	21 (78)	0.10
Neonates with Rhesus c, n (%)	13 (8)	4 (15)	0.29
Neonates with Rhesus C, n (%)	2 (1)	0 (0)	1.00
Neonates with Rhesus E, n (%)	1 (1)	2 (7)	0.06

[a] Value given as median (IQR), [b] Value given as mean ± SD

The use of IUT, phototherapy and ET

Detailed information on the use of IUT is presented in Table 1 and on the use of phototherapy and ET in group I and group II is presented in Table 2. Serial IUTs were performed in 99 (63%) infants in group I and 15 (56%) in group II (p = 0.52). At least one ET was required in 66% (103/156) of the patients in group I compared to 26% (7/27) of the patients in group II (p = <0.01). The rate of ET performed on day 1 dropped from 96% (94/103) in group I to 57% (4/7) in group II (p = <0.01). The median number of ETs was 1 in group I (IQR 0-1, range 0-5) and 0 in group II (IQR 0-1, range 0-2) (p = <0.01). No significant relationship was found between the number of IUTs and the number of ETs (Spearman correlation coefficient = -0.009; p = 0.90).

The use of top-up transfusions

Complete information on the number of top-up red cell transfusions was obtained for 98% (180/183) of neonates. Detailed information on the use of top-up transfusions in group I and group II is presented in Table 3. The percentage of neonates in group I and group II receiving a top-up transfusion was 68% (105/154) and 81% (22/27), respectively (p = 0.25). The median number of top-up transfusions per infant in group I and II was 1 (IQR 0-2, range 0-6) and 2 (IQR 1-3, range 0-5) (p = 0.01).

Combining group I and II, we found a negative correlation between the number of ETs and the number of top-up blood transfusions (Spearman correlation coefficient -0.183; p = 0.01). This correlation between a lower number of ETs and a higher number of top-up transfusions was present mainly in the subgroup of neonates treated with IUT (n = 114) (Spearman correlation coefficient = -0.340; p = <0.01). In the subgroup of neonates without IUT (n = 69), the correlation between the number of ETs and the number of top-up blood transfusions was not significant (Spearman correlation coefficient = 0.011; p = 0.93). Of the 110 neonates that received an ET 73 neonates (66%) were treated with IUT.

Table 2. Neonatal outcome in group I and group II

	Group I (n = 156)	**Group II** (n = 27)	**p-value**
Hemoglobin level at birth, g/dL [a]	11.6 ± 2.58	12.6 ± 3.40	0.25
Bilirubin level at birth, mg/dL[a]	6.0 ± 2.70	6.2 ± 2.85	0.89
Reticulocyte count at birth, ‰[b,c]	21 (3 - 61)	47.5 (3 - 106)	0.19
Maximum bilirubin, mg/dL[b]	13.6 (10.3 – 16.8)	15.4 (13.2 -18.0)	0.03
Neonates treated with phototherapy, n (%)	150 (96)	27 (100)	0.59
Phototherapy, days[a]	4.1 ± 1.88	4.7 ± 1.59	0.11
Neonates treated with ET, n (%)	103 (66)	7 (26)	<0.01
Number of ETs per neonate[b]	1 (0 -1)	0 (0 -1)	<0.01
ETs performed on day 1, n (%)	94 (96)	4 (57)	<0.01
Duration of admission at our center, days[a]	6.0 ± 3.3	6.3 ± 3.9	0.47

[a] Value given as mean ± SD, [b] Value given as median (IQR), [c] Assessed in 75 and 20 patients of group I and II, respectively

Table 3. Top-up transfusions in neonates with Rhesus hemolytic disease in group I and group II

	Group I (n = 156)	**Group II** (n = 27)	**p-value**
Neonates receiving top-up transfusions, n (%)	105/154 (68)	22/27 (81)	0.25
Number of top-up transfusions per infant[a]	1 (0-2)[c]	2 (1-3)	0.01
Neonates receiving: 1 top-up transfusion, n (%)	41 (39)	3 (14)	0.08
2 top-up transfusions, n (%)	37 (36)	9 (41)	0.31
3 top-up transfusions, n (%)	11 (11)	6 (27)	0.03
4 top-up transfusions, n (%)	11 (11)	3 (14)	0.44
5 top-up transfusions, n (%)	2 (2)	1 (4)	0.39
6 top-up transfusions, n (%)	2 (2)	0 (0)	1.00
Days after birth until first top-up transfusion[a]	19.5 (1 - 34.8)[c]	10 (2.5 – 23.0)[d]	0.23
Hemoglobin level at first top-up transfusion, g/dL[b]	8.3 ± 1.5[e]	8.2 ± 1.3[f]	0.72

[a] Value given as median (IQR) , [b] Value given as mean ± SD, [c] Assessed in 153/156 neonates, [d] Assessed in 17/22 neonates, [e] Assessed in 95/105 neonates, [f] Assessed in 16/22 neonates

Discussion

This study shows that after implementation of a more restrictive guideline for ET in neonates with RHD, the rate of ETs decreased considerably from 66% to 26%. The reduction in ET rate was associated with a significant increase in the number of top-up transfusions for neonatal anemia. Neonates in group II required twice more top-up transfusions than neonates in group I. Since the criteria for top-up transfusions remained unchanged before and after the ET guideline changes, our findings suggest that neonatal anemia in neonates with RHD is more likely to occur if restrictive ET criteria are used.

Our findings are in accordance with a previous study by al-Alaiyan et al. in a small group of 36 (near-) term neonates with RHD treated with IUT.[3] Al-Alaiyan et al. reported a similar association between ET rate and top-up transfusions.[3] The risk of neonatal anemia in neonates treated with and without ET was 36% and 92%, respectively ($p = 0.04$).[3]

The reduced rate of top-up transfusions in infants treated with ET can be attributed to the removal of antibodies and IgG coated erythrocytes during ET, hence reducing the hemolytic process and the risk of neonatal anemia.[3,6] However, the effect of ET on the antibody titer on the short term is limited. Since the antibodies are distributed in the intravascular as well as the extravascular fluid, ET reduces the antibody titer only by about 25 percent.[4,10] The beneficial effect of ET lies in replacing nearly all of the Rhesus positive red cells by immunologically compatible cells, hence surviving cells in the blood stream.[4]

In contrast with previous studies[2,8] we found a significant negative correlation between the number of ETs and top-up transfusions. This negative correlation was found only in neonates treated with IUT, but was not found in neonates without IUT treatment. Since fetuses requiring IUT treatment are more severely affected by RHD than fetuses without IUT treatment, we speculate that the beneficial effect of ET in reducing the number of top-up transfusions occurs primarily in severely affected neonates with high titer antibodies. Washing out antibodies and replacing Rhesus positive cells through ET treatment may be particularly more effective in severely affected neonates.

The pathogenesis of neonatal or late anemia in RHD is not completely clarified and can be due to either depressed erythropoiesis ("hyporegenerative anemia") or persisting reduction in half-life of the Rhesus positive erythrocytes caused by remaining antibodies ("late anemia of hemolytic disease") if age-appropriate or elevated reticulocytes are present.[11,12] "Hyporegenerative anemia" occurs in particular after IUT due to suppression of the erythropoiesis.[2,9] An alternative explanation for failing compensatory reticulocytosis is destruction of bone marrow precursors and reticulocytes by antibodies.[4,13] Other contributing factors to neonatal anemia are reduced survival of transfused red blood cells[5],

natural decline of the hemoglobin level toward the physiological nadir, and the increasing intravascular volume of the growing neonate.[1] Finally, erythropoietin deficiency can be a possible contributing factor to neonatal anemia.[12] Treatment with erythropoietin has been suggested to reduce the number of top-up transfusions, but the evidence to recommend routine use of erythropoietin is very limited.[14]

The data in this study should be interpreted with care due to the retrospective design of the study which may have led to a selection bias and influenced by changing transfusion attitudes over time. Although both groups were similar in terms of severity of fetal anemia, need of IUT, and hemoglobin levels at birth, it is conceivable that neonates in group II were more severely affected than neonates in group I, hence requiring more top-up transfusions.[2] Importantly, the percentage of male infants in group II was higher than in group I. Male infants have a higher prevalence of HDN secondary to Rhesus alloimmunization,[15] a higher neonatal death rate from kernicterus[16] and appear to be more severely affected than females in terms of need for IUT, development of hydrops fetalis and perinatal mortality.[17] A larger study is required to determine if this sex difference in baseline characteristic may have influenced our results.

In conclusion, this study shows that the number of ETs in neonates with HDN secondary to Rhesus alloimmunization decreased significantly after the introduction of restrictive ET criteria. Reduction of ET rate resulted in a doubling of the number of top-up transfusions. Restrictive ET criteria in HDN secondary to Rhesus alloimmunization during the neonatal period may thus lead to an increased rate of neonatal anemia. Nevertheless, the risk of adverse events associated with ETs (in particular catheter-related complications) is high (7%)[18] compared to the transfusion-related risks of blood transfusion in general (<0.04%).[19] A reduction in ETs, despite an increase in top-up transfusions may therefore be more beneficial for neonates with HDN secondary to Rhesus alloimmunization, although the long-term effects on neurodevelopmental outcome requires longer follow-up.

References

1. Pessler F, Hart D: Hyporegenerative anemia associated with Rh hemolytic disease: treatment failure of recombinant erythropoietin. J Pediatr Hematol Oncol 2002; 24: 689-693.
2. 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.
3. al-Alaiyan S, al Omran A: Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med 1999; 27: 112-115.
4. Giblett ER, Varela JE, Finch CA: Damage of the bone marrow due to Rh antibody. Pediatrics 1956; 17: 37-44.
5. Koenig JM, Ashton RD, De Vore GR, Christensen RD: Late hyporegenerative anemia in Rh hemolytic disease. J Pediatr 1989; 115: 315-318.
6. 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):265-71.
7. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004; 114: 297-316.
8. Ebbesen F: Late anaemia in infants with rhesus haemolytic disease treated with intensive phototherapy. Eur J Pediatr 1979; 130: 285-290.
9. 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.
10. Diamond LK, Allen FH, Jr., Vann DD, Powers JR: Erythroblastosis fetalis. Pediatrics 1952; 10: 337-347.
11. Hayde M, Widness JA, Pollak A, Kohlhauser-Vollmuth C, Vreman HJ, Stevenson DK: Rhesus isoimmunization: increased hemolysis during early infancy. Pediatr Res 1997; 41: 716-721.
12. Millard DD, Gidding SS, Socol ML, MacGregor SN, Dooley SL, Ney JA, Stockman JA, III: Effects of intravascular, intrauterine transfusion on prenatal and postnatal hemolysis and erythropoiesis in severe fetal isoimmunization. J Pediatr 1990; 117: 447-454.
13. Burk CD, Malatack JJ, Ramsey G: Misleading Rh phenotype and severe prolonged anemia in hemolytic disease of the newborn. Am J Dis Child 1987; 141: 712-713.
14. 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.
15. Woodrow JC, Donohoe WT: Rh-immunization by pregnancy: results of a survey and their relevance to prophylactic therapy. Br Med J 1968; 4: 139-144.
16. Walker W, Mollison PL: Haemolytic disease of the newborn; deaths in England and Wales during 1953 and 1955. Lancet 1957; 272: 1309-1314.
17. Ulm B, Svolba G, Ulm MR, Bernaschek G, Panzer S: Male fetuses are particularly affected by maternal alloimmunization to D antigen. Transfusion 1999; 39: 169-173.
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. Stainsby D, Jones H, Wells AW, Gibson B, Cohen H: 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: 73-79.

Chapter 4

Neonatal morbidity after exchange transfusion for red cell alloimmune hemolytic disease

Mirjam E.A. Rath & Vivianne E.H.J. Smits-Wintjens (contributed equally)
Erik W. van Zwet
Dick Oepkes
Anneke Brand
Frans J. Walther
Enrico Lopriore

Neonatology; 2013;103:141-147

Abstract

Background

Exchange transfusion (ET) is a high risk procedure. Type and rate of complications in neonatal red cell alloimmune hemolytic disease exclusively is not clear.

Objective

Our aim was to study type and rate of complications associated with ET in a large series of neonates with hemolytic disease of the fetus and newborn (HDFN) due to red cell alloimmunization.

Methods

All neonates with HDFN due to red cell alloimmunization admitted to our center between January 2001 and June 2011 were eligible for this study. We recorded the number and rate of complications during admission in the group of neonates treated with (ET-group) and without ET (no-ET-group). Multivariate logistic regression analysis was performed to measure independent risk of complications of ET treatment.

Results

A total of 347 infants with red cell alloimmune hemolytic disease were included, 39% (134/347) was treated with at least one ET (ET-group) and 61% (213/347) did not require ET (no-ET-group). Comparison between ET-group and no-ET-group showed that ET treatment was independently associated with: proven sepsis (8% versus 1% respectively, odds ratio (OR) 8.3, 95% confidence interval (CI) 1.7-40.3, p = 0.009), leukocytopenia (88% versus 23% respectively, OR 36.0, 95% CI 17.5-73.8, p = <0.001), severe thrombocytopenia (platelet count $<50 \times 10^9$/L) (63% versus 8% respectively, OR 31.4, 95% CI 14.0-70.4, p = <0.001), hypocalcemia (22% versus 1% respectively, OR 27.4, 95% CI 5.9-126.8, p = <0.001) and hypernatremia (8% versus 0% respectively, p = <0.001). Neonatal death did not occur in the ET-group.

Conclusion

ET in neonates with HDFN is associated with increased risk of sepsis, leukocytopenia, thrombocytopenia, hypocalcemia and hypernatremia.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) due to red cell alloimmunization may lead to excessive unconjugated hyperbilirubinemia. Neonatal treatment consists of intensive phototherapy and exchange transfusion (ET) to prevent kernicterus. After introduction in the late 1940s[1] neonatal treatment with ET became one of the most commonly performed procedures. However, ET is a high-risk invasive procedure requiring the use of central lines and is associated with a significant rate of adverse events. Although the contemporary mortality rate is reported to be less than 2%, rates of morbidity and ET-related adverse events can reach 74%.[2-9] Reported adverse events include catheter-related complications, complications related to the use of blood products, metabolic derangements and cardio-respiratory reactions.[2-9]

In nearly all previous studies a heterogeneous group of infants with HDFN treated with ET was included, varying from HDFN due to red cell alloimmunization to HDFN due to ABO-incompatibility.[2-4,6,8,9] Hemolysis caused by ABO-incompatibility is usually less severe compared to red cell alloimmunization and therefore associated with a reduced rate of neonatal morbidity. Furthermore, different indications for ET were used[2,8,9] and most studies were limited by a relative small number of included patients or were not case-controlled.[2-9] The aim of this study was to evaluate type and rate of complications associated with ET in a large series of neonates with HDFN due to red cell alloimmunization exclusively.

Patients and Methods

All term and preterm neonates with HDFN due to maternal red cell alloimmunization treated with or without ET, admitted to our center between January 2001 and June 2011 were eligible for this retrospective observational study. The Leiden University Medical Center (LUMC) is the national referral center for the management and intrauterine treatment of HDFN in the Netherlands. Neonatal outcome in part of this group was reported in previous studies.[10-14] In the Netherlands no formal ethical approval by the Medical Ethics Committee or written informed consent is necessary for retrospective studies.

Guidelines for ET used in our neonatal center were revised in December 2005.[10,11] Before December 2005, criteria for ET included: (1) bilirubin level at birth >3.5 mg/dL and/or (2) total serum bilirubin level above ET thresholds (rise of bilirubin value >0.5 mg/dL/h despite intensive phototherapy). In neonates not treated with IUT, a hemoglobin level at birth of <12.9 g/dL was also considered as a criterion for ET. Criteria for ET after December 2005

were: (1) total serum bilirubin above (higher) ET thresholds and/or (2) rise of bilirubin >0.5 mg/dL/h despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level.[15]
ET was performed with double-volume transfusion (160 mL/kg) with citrated plasma (citrate 12-19 mEq/L) from a non-transfused male donor, lacking irregular erythrocyte-antibodies, added to irradiated, leukocyte-depleted (<1 × 10^6/L) and thrombocyte-reduced red cell concentrate, compatible with maternal antibodies. Citrated plasma contains nearly no free calcium, physiologic levels of potassium and glucose and an increased concentration of sodium (168 mEq/L) (Sanquin Bloodbank, The Netherlands). According to our guidelines no standard calcium supplementation is used during ET.
Primary outcome of this study was the rate of complications during admission in the group treated with ET (ET-group) and without ET (no-ET-group).

The following adverse events (complication occurring during admission) were recorded: hypocalcemia (<8 mg/dL), hypoglycemia (<46.8 mg/dL), hyperkalemia (>6.5 mEq/L), hypokalemia (<3.0 mEq/L), hypernatremia (>150 mEq/L), hyponatremia (<130 mEq/L), metabolic acidosis (pH <7.25, bicarbonate <22 mEq/L requiring treatment with bicarbonate), respiratory failure (respiratory support with continuous positive airway pressure and/or mechanical ventilation), apnea (cessation of respiration >20 seconds), pulmonary hemorrhage, cardiac arrest, hypertension (requiring antihypertensive medication), hypotension (requiring intravenous fluids or vasopressors), necrotizing enterocolitis,[16] proven sepsis (clinical and/or biochemical signs of infection (leukocytes <24 hours after birth <9 × 10^9/L and >24 hours <5 × 10^9/L or leukocytes >25 × 10^9/L and/or band forms >20% of leukocytes and/or C reactive protein >10mg/L) with positive blood culture), suspected sepsis (clinical and/or biochemical signs of infection without positive blood culture), disseminated intravascular coagulation (DIC) (requiring fresh frozen plasma), seizures (treated with anti-epileptic medication), leukocytopenia (<24 hours after birth <9 × 10^9/L and >24 hours <5 × 10^9/L) thrombocytopenia (<150 × 10^9/L; severe if platelet count <50 × 10^9/L and very severe if platelet count <20 × 10^9/L), intraventricular hemorrhage[17], or other cerebral hemorrhage and neonatal death. Platelet transfusions are given when platelets are: 1) <100 × 10^9/L before planned ET and 2) <50 × 10^9/L after ET.

We recorded the following obstetric and neonatal data: type of red cell alloimmunization, number of intrauterine red cell transfusions (IUT), gestational age at birth, birth weight, presence of hydrops at birth, hemoglobin level, reticulocyte count, leukocyte count and bilirubin level at birth, days of admission on our neonatal intensive care unit, occurrence

of complications during admission and time of occurrence in relation to ET, number of ETs and presence of umbilical venous catheter (UVC). Before ET-guideline change in 2005 a UVC was inserted directly after birth in all neonates. After 2005 a UVC was inserted when bilirubin levels increased towards ET-threshold. UVCs were left in place until bilirubin levels were decreased towards or below phototherapy threshold. According to our guidelines ultrasound to detect portal vein thrombosis (PVT) after ET was not performed standard but only in the presence of clinical signs of thrombosis.

Data are reported as means and standard deviations (SD) or as medians and interquartile ranges (IQR). Statistical analysis was performed using Student-t test and Mann-Whitney test for continuous variables. Chi-square and Fisher's-exact test were used for categorical variables. A p-value <0.05 was considered to indicate statistical significance. Of all statistically significant complications identified with univariate analysis a multivariate logistic regression analysis was performed to measure independent effect of ET(s). Results of logistic regression models were expressed as odds ratios (OR) and 95% confidence intervals (CI). Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

During this 10-year study, 348 neonates with HDFN due to red cell alloimmunization were admitted to our nursery. We excluded one preterm neonate (29 weeks' gestation) because he died immediately after caesarean section due to severe perinatal asphyxia following unsuccessful IUT. 347 Patients were included, 134 (39%) in the ET-group and 213 (61%) in the no-ET-group. Baseline characteristics are summarized in Table 1. The total number of ETs in the ET-group was 207 and the median (range) number of ETs was 1 (1-9).
In the ET-group 93/134 (69.4%) neonates needed one or more top-up transfusions compared to 162/213 (76.1%) in the no-ET-group.

Complications
Detailed information on complications during admission in both groups is summarized in Table 2.

Table 1. Baseline characteristics in ET-group and no-ET-group

	ET (n = 134)	**no-ET** (n = 213)	**p-value**
Type of red cell alloimmunization			
Rh D, n (%)	122 (91)	138 (65)	<0.001
Rh c, n (%)	11 (8)	16 (8)	0.813
Kell, n (%)	1 (1)	46 (22)	<0.001
Other types than Rh D, Rh c or Kell, n (%)	0 (0)	13 (6)	
Neonates treated with IUT, n (%)	99 (74)	141 (66)	0.131
Number of IUTs per neonate[a]	2 (2-3)	3 (2-4)	0.009
Gestational age at first IUT, weeks[a]	30 (25-33)	26 (23-31)	0.001
Birth weight, kg[b]	2.9 ± 0.6	2.9 ± 0.6	0.591
Gestational age at birth, weeks[a]	36 (36-37)	36 (36-37)	0.247
Male, n(%)	89 (66)	130 (61)	0.311
Hydrops at birth, n (%)	2 (2)	4 (2)	1.000
Hemoglobin level at birth, g/dL[b]	11.6 ± 2.6	12.7 ± 2.9	<0.001
Reticulocyte count at birth, ‰[a,c]	49 (6.8-83.3)	39 (3-75.5)	0.089
Leukocyte count at birth, 10^9/L[b,d]	14.1 ± 5.6	13.4 ± 5.7	0.270
Thrombocytopenia at birth, n (%)	39 (29)	48 (23)	0.169
Bilirubin level at birth, mg/dL[b]	7.1 ± 3.1	4.8 ± 2.3	<0.001
Umbilical venous catheter, n (%)	133 (99)	64 (30)	<0.001
Days of admission on NICU[b]	6.6 ± 3.8	6.2 ± 3.9	0.009

[a] Value given as median (IQR), [b] Value given as mean ± SD, [c] assessed in 78/134 and 149/213 neonates, [d] assessed in 130/134 and 207/213 neonates, NICU = neonatal intensive care unit

Univariate analysis

Metabolic derangements/complications

Hypocalcemia (22% versus 1%) and hypernatremia (8% versus 0%) occurred significantly more in the ET-group. Four of 31 (13%) neonates with hypocalcemia needed calcium supplementation and 3/11 (27%) needed treatment (additional sodium-free intravenous fluid) for hypernatremia. Severe symptoms of hypernatremia (seizures) did not occur.

Cardio-respiratory complications

No significant differences were seen in respiratory support, apneas, cardiac arrest and hypotension (Table 2). No cases of cardiac rhythm disorders, pulmonary hemorrhage and hypertension occurred.

Table 2. Complications during admission in ET-group and no-ET-group

			univariate analysis		multivariate analysis	
	ET-group (n = 134)	**no-ET-group** (n = 213)	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Hypocalcemia	29 (21.6)	2 (0.9)	29.1 (6.8-124.5)	<0.001	27.4 (5.9-126.8)	<0.001
Hypoglycemia	14 (10.4)	28 (13.1)		0.453		0.848
Hyperkalemia	1 (0.7)	0		0.386		NC
Hypokalemia	3 (2.2)	1 (0.5)		0.303		0.220
Hypernatremia	11 (8.2)	0	NC	<0.001	NC	NC
Hyponatremia	1 (0.7)	2 (0.9)		1.000		0.949
Metabolic acidosis	2 (1.5)	7 (3.3)		0.491		0.103
Respiratory support	17 (12.7)	18 (8.5)		0.202		0.425
Apneas	7 (5.2)	3 (1.4)		0.050		0.137
Cardiac arrest	0	2 (0.9)		0.525		NC
Hypotension	2 (1.5)	4 (2)		1.000		0.356
NEC	1 (0.7)	2 (1.9)		1.000		1.000
Proven sepsis	11 (8.2)	3 (1.4)	6.3 (1.7-22.9)	0.002	8.3 (1.7-40.3)	0.009
Suspected sepsis	9 (6.7)	16 (7.5)		0.780		0.466
Leukocytopenia	118 (88.1)	49 (23.0)	24.7 (13.4-45.5)	<0.001	36.0 (17.5-73.8)	<0.001
Thrombocytopenia	132 (98.5)	67 (31.5)	143.8 (34.6-598.6)	<0.001	146.9 (34.3-629.1)	<0.001
Severe	85 (63.4)	16 (7.5)	21.4 (11.5-39.7)	<0.001	31.4 (14.0-70.4)	<0.001
Very severe	14 (10.4)	3 (1.4)	8.2 (2.3-29.0)	0.001	11.5 (2.5-53.2)	0.002
DIC	0	1 (0.5)		1.000		0.982
Seizures	2 (1.5)	1 (0.5)		0.562		0.931
Death	0	1 (0.5)		1.000		NC

Values in columns 2 and 3 represent n (%). Respiratory support = continuous positive airway pressure and/or mechanical ventilation, NEC = necrotizing enterocolitis, DIC = disseminated intravascular coagulation, NC = not calculated

Infectious complications

Proven sepsis and leukocytopenia occurred significantly more often in the ET-group (8% versus 1% and 88% versus 23%, respectively). All ET-treated neonates with proven sepsis had leukocytopenia during admission and in 55% (6/11) leukocytopenia occurred after ET. In the 14 neonates from both groups with proven sepsis, bacterial cultures were positive for Staphylococcus aureus (7/14), coagulase-negative Staphylococcus (3/14), beta-hemolytic Streptococcus (1/14), Klebsiella pneumonia (1/14), Escherichia coli (1/14) and Bacillus cereus (1/14). This last patient developed a sepsis with brain abscesses after an ET performed through a UVC.[18]

Hematological complications

The rate of thrombocytopenia was significantly higher in the ET-group (99% versus 32%). Seventy-five of 134 ET-treated neonates were treated with at least one platelet transfusion. One near-term neonate (36 weeks' gestation) received a platelet transfusion after ET on day one because of post-ET platelet count 39 × 10^9/L. On day 2 cranial ultrasonography showed a hemorrhage in the right parieto-occipital periventricular white matter, which was not observed antenatally. Magnetic resonance imaging (MRI) showed no sinus thrombosis and coagulation was normal. At one year of age, the infant had no neurologic sequelae. In the no-ET-group 3 neonates had intracerebral hemorrhage on cranial ultrasound and/or MRI of whom one neonate had severe thrombocytopenia for which he received 4 platelet transfusions. This neonate died during admission (see below).
In both study-groups no cases of bilirubin encephalopathy or PVT were observed.

Neonatal mortality

Only one of 347 neonates died during admission. This neonate from the no-ET-group (30 weeks' gestation) had severe fetal hydrops. On day 2 bilirubin levels increased above ET threshold, however due to intravenously access problems ET could not be performed. Subsequently, bilirubin levels decreased below ET threshold with phototherapy. He died on day 11 due to respiratory failure, pulmonary hypertension, bilateral intraventricular hemorrhage grade 2 and renal failure.

ET guideline change

To measure the possible effect of ET guideline change[10] on the rate of the previously mentioned complications we performed a sub-analysis of ET-treated neonates before and after guideline change. Rates of complications were not significantly different between both groups (data not shown).

Multivariate analysis

Complications during entire admission

We corrected for the following covariates: hemoglobin level at birth, type of red cell alloimmunization, days of admission and gestational age at birth. We corrected for the first two because of significant differences at baseline (Table 1) and for the latter because preterm neonates are more susceptible for ET-related complications.[8,19] Since the presence of UVC and ET are correlated (Spearman correlation coefficient r = 0.680, p = <0.001), we did not correct for the presence of a UVC. Proven sepsis (OR 8.3), severe thrombocytopenia (OR 31.4), leukocytopenia (OR 36.0) and hypocalcemia (OR 27.4), had a higher incidence in the ET-group.

Complications after first ET

Since our main interest is in ET-related complications, we performed a sub-analysis excluding all complications in the ET-group that were observed before (first) ET (adjusted-ET-group, Table 3). Similar to the entire data set, neonates undergoing ET were more likely to experience sepsis, leukocytopenia, thrombocytopenia, hypocalcemia and hypernatremia.

Table 3. Complications during admission in Adjusted Exchange Transfusion (ET) and No Exchange Transfusion group

			Univariate analyses		Multivariate analyses	
	Adjusted ET group n = 134	**No ET group** n = 213	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Hypocalcemia	25 (18.7)	2 (0.9)	24.2 (5.6-104.1)	<0.001	21.9 (4.7-101.7)	<0.001
Hypernatremia	10 (7.5)	0	NC	<0.001	NC	NC
Proven sepsis	8 (6.0)	3 (1.4)	4.4 (1.6-17.1)	0.030	5.30 (1.0-27.1)	0.046
Leukocytopenia	91 (70.5)[b]	49 (23.0)	8.0 (4.9-13.2)	<0.001	9.0 (5.1-15.9)	<0.001
Thrombocyto-penia	90 (67.2)	67 (31.5)	4.5 (2.8-7.1)	<0.001	3.90 (2.4-6.4)	<0.001
Severe	71 (53)	16 (7.5)	14.9 (8.0-27.8)	<0.001	16.3 (7.8-34.1)	<0.001
Very severe	14 (10.4)	3 (1.4)	8.2 (2.3-29.0)	<0.001	11.5 (2.5-53.1)	0.002

Values in columns 2 and 3 represent n (%). For the adjusted ET group, only complications which occurred after first ET were analyzed. [b] Assessed in 129/134 neonates

Discussion

This study demonstrates that treatment with ET in neonates with HDFN is associated with an increased risk of sepsis, thrombocytopenia, leukocytopenia, hypocalcemia and hypernatremia. Treatment with ET was not associated with neonatal death in our cohort. Several studies have been published on neonatal morbidity due to treatment with ET.[2-9] One of the known risk factors associated with ET is development of invasive bacterial infections. The reported incidence of ET-related sepsis ranges from 0% to 11%.[4,6,8] The incidence of proven sepsis detected in this study (8%) is in accordance with these previous reports.
We found that the independent risk of sepsis was more than eight times higher in the ET-group. The exact cause of increased risk of infection is not fully understood, but is most probably related to the use of UVCs for ET. UVCs are a well-known risk factor for nosocomial infection.[20] Sepsis in the ET-group may also be caused by administration of infected blood products. Although the current risk of transmission of infectious diseases is relatively low, it is not completely negligible.[5,21] Furthermore, ET-related wash out of leukocytes may also play a role in the higher incidence of sepsis in the ET-group, since in double volume ET more than 90% of circulating blood is replaced by leukocyte-depleted donor-blood.[21,22] Finally, limited data on leukocytopenia in neonates with HDFN due to red cell alloimmunization show that the risk of leukocytopenia is increased in severe Rhesus HDFN.[23,24] In this study 86% (12/14) of neonates with proven sepsis had leukocytopenia of whom 11 were treated with ET. In 55% (6/11) leukocytopenia occurred after treatment with ET. In the remaining 45% leukocytopenia might be caused by bone marrow suppression due to increased erythropoiesis.
Another important ET-related complication is PVT. Risk factors for PVT include umbilical catheterization and ET.[25] However, none of the included infants in our study was diagnosed with PVT, probably because in this retrospective study no standard ultrasound to detect PVT after umbilical catheterization and/or ET has been performed.
Another reported risk associated with ET is thrombocytopenia due to the use of a thrombocyte-free blood product.[26] Previous studies reported incidences of ET-related thrombocytopenia ranging from 6% to 44%.[2-4,6,8,9] In our study, the incidence of thrombocytopenia in the ET-group was much higher, almost all neonates (99%) had low platelet counts and 63% had severe thrombocytopenia. Differences in incidence can be explained by differences in methodology and study population. We only included neonates with red cell alloimmune HDFN which is known to be associated with an increased risk for thrombocytopenia, even without ET.[14,27,28] Because other studies included neonates with ABO-incompatibility, their incidence of thrombocytopenia should be lower. In this study,

the risk of severe thrombocytopenia is 21-fold higher in the ET-group. Neonatologists must be aware of this potentially devastating complication as massive hemorrhage may arise after ET. Complications can be prevented by prophylactic platelet transfusion. In our study, platelet transfusions were administered in 57% of neonates before, during or after ET.
A further complication of ET is hypocalcemia, resulting from the use of citrated blood which contains almost no free calcium.[26,29] Previous studies reported incidences ranging from 3% to 42%.[4,8,10,11] In our study, 22% of neonates in the ET-group had hypocalcemia, and 13% needed replacement therapy. Treatment with ET was independently associated with an almost 30-fold increased risk of hypocalcemia. If left untreated, hypocalcemia can lead to potentially devastating complications such as seizures and cardiac arrhythmias. It is therefore crucial to measure calcium levels during the ET-procedure and act accordingly.
Another metabolic complication related to ET is hypernatremia. Hypernatremia probably results from an increased level of sodium in citrated blood. Only few studies have reported on hypernatremia resulting from ET, and the exact incidence is unknown.[30,31] Because hypernatremia can lead to serious complications we recommend frequent measurements of serum sodium levels during and after ET.
Finally, previous studies have reported that ET may also lead to neonatal death. Neonatal mortality attributable to ET ranges between 0.5% and 2%.[2,6-8] In accordance with our findings, three recent studies reported no ET-related deaths.[3,4,9] However, these studies (as well as ours) were not powered to detect a difference in neonatal mortality. Differences between reported rates can be explained by methodological differences such as different sizes of study cohorts and differences in disease-severity between cohorts. Neonates included in previous studies were often more premature than our study-population.[7-9]
Another explanation could be that our center is the national referral center for intrauterine treatment of red cell alloimmunization. Consequently all severely affected neonates with HDFN are born and treated in our center. As a result, ET is a frequently performed and standardized procedure and part of routine practice. We speculate that this may have contributed to the low level of severe morbidity and mortality in the ET-group. Operator's experience ('learning curve') could also be a factor in the reduction of morbidity and mortality in the ET-group.
Future alternative treatments for hyperbilirubinemia might theoretically further reduce the rate of ET and consequently decrease morbidity and mortality rates.[32]

In conclusion, sepsis, leukocytopenia, thrombocytopenia, hypernatremia and hypocalcemia are common complications in neonates with HDFN due to red cell alloimmunization treated with ET. In experienced hands severe permanent morbidity and mortality rates due to ET-procedures can be reduced to a minimum.

References

1. Diamond LK, Allen FH, Jr., Thomas WO, Jr: Erythroblastosis fetalis. VII. Treatment with exchange transfusion. N Engl J Med 1951; 244:39-49.
2. Badiee Z: Exchange transfusion in neonatal hyperbilirubinaemia: experience in Isfahan, Iran. Singapore Med J 2007; 48:421-3.
3. Davutoglu M, Garipardic M, Guler E, Karabiber H, Erhan D: The etiology of severe neonatal hyperbilirubinemia and complications of exchange transfusion. Turk J Pediatr 2010; 52:163-6.
4. Hosseinpour SS, Gharehbaghi MM: Exchange transfusion in severe hyperbilirubinemia: an experience in northwest Iran. Turk J Pediatr 2010; 52:367-71.
5. Ip S, Chung M, Kulig J, O'Brien R, Sege R, Glicken S et al: An evidence-based review of important issues concerning neonatal hyperbilirubinemia. Pediatrics 2004; 114:e130-e153.
6. Jackson JC: Adverse events associated with exchange transfusion in healthy and ill newborns. Pediatrics 1997; 99:E7.
7. Keenan WJ, Novak KK, Sutherland JM, Bryla DA, Fetterly KL: Morbidity and mortality associated with exchange transfusion. Pediatrics 1985; 75:417-21.
8. Patra K, Storfer-Isser A, Siner B, Moore J, Hack M: Adverse events associated with neonatal exchange transfusion in the 1990s. J Pediatr 2004; 144:626-31.
9. 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.
10. Rath ME, Smits-Wintjens VE, Lindenburg I, Brand A, Oepkes D, Walther FJ et al: Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010; 99:65-70.
11. Rath ME, Smits-Wintjens VE, Lindenburg IT, Brand A, Van Kamp IL, Oepkes D et al: Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011; 100:312-6.
12. 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.
13. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM et al: Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011; 127:680-6.
14. Rath ME, Smits-Wintjens VE, Oepkes D, van Zwet EW, Van Kamp IL, Brand A et al: Thrombocytopenia at birth in neonates with red cell alloimmune haemolytic disease. Vox Sang 2012; 102(3):228-33.
15. 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.
16. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L et al: Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg 1978; 187:1-7.
17. Volpe JJ: Intracranial hemorrhage: germinal matrix-intraventricular hemorrhage of the premature infant. In: Volpe JJ, editor. Neurology of the newborn. Philadelphia: Saunders; 2001. p. 428-93.

18. Smits-Wintjens VE, Steggerda SJ, Oepkes D, Van Kamp IL, Kramer CM, Walther FJ et al: Bacillus cereus cerebral abscesses in a term neonate with rhesus hemolytic disease treated with exchange transfusion. J Pediatr Inf Dis 2010; 5:277-80.
19. Maisels MJ, Watchko JF: Treatment of jaundice in low birthweight infants. Arch Dis Child Fetal Neonatal Ed 2003; 88:F459-F463.
20. Inglis GD, Davies MW: Prophylactic antibiotics to reduce morbidity and mortality in neonates with umbilical venous catheters. Cochrane Database Syst Rev 2005;CD005251.
21. Fergusson D, Hebert PC, Barrington KJ, Shapiro SH: Effectiveness of WBC reduction in neonates: what is the evidence of benefit? Transfusion 2002; 42:159-65.
22. Xanthou M, Nicolopoulos D, Gizas A, Matsaniotis N: The response of leukocytes in the peripheral blood during and following exchange transfusion in the newborn. Pediatrics 1973; 51:570-4.
23. Rath ME, Smits-Wintjens VE, Walther FJ, Lopriore E: Hematological morbidity and management in neonates with hemolytic disease due to red cell alloimmunization. Early Hum Dev 2011; 87:583-8.
24. Blanco E, Johnston DL: Neutropenia in infants with hemolytic disease of the newborn. Pediatr Blood Cancer 2011; doi:10.1002/pbc.23233.
25. Williams S, Chan AK: Neonatal portal vein thrombosis: diagnosis and management. Semin Fetal Neonatal Med 2011; 16:329-39.
26. Samsom JF, Groenendijk MG, van der Lei J, Okken A: Exchange transfusion in the neonate, a comparison between citrate-, heparinized- and reconstituted whole blood. Eur J Haematol 1991; 47:153-4.
27. Saade GR, Moise KJ, Jr., Copel JA, Belfort MA, Carpenter RJ, Jr: Fetal platelet counts correlate with the severity of the anemia in red-cell alloimmunization. Obstet Gynecol 1993; 82:987-91.
28. 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; 199:387-4.
29. Maisels MJ, Li TK, Piechocki JT, Werthman MW: The effect of exchange transfusion on serum ionized calcium. Pediatrics 1974; 53:683-6.
30. Doyle PE, Eidelman AI, Lee K, Daum C, Gartner LM: Exchange transfusion and hypernatremia: possible role in intracranial hemorrhage in very-low-birth-weight infants. J Pediatr 1978; 92:848-9.
31. Steele AM, Brown DL, Lipsitz PJ: Relationship of exchange transfusion to hypernatremia. J Pediatr 1979; 94:168-9.
32. Smits-Wintjens VEHJ, Walther FJ, Lopriore E: Rhesus hemolytic disease of the newborn: postnatal management, associated morbidity and long-term outcome. Semin Fetal Neonatal Med 2008; 13:265-71.

Part 2

Intravenous immunoglobulin

Chapter 5

Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial

Vivianne E.H.J. Smits-Wintjens
Frans J. Walther
Mirjam E.A. Rath
Irene T.M. Lindenburg
Arjan B. te Pas
Christine M. Kramer
Dick Oepkes
Anneke Brand
Enrico Lopriore

Pediatrics 2011; 127:680-686

Abstract

Background

Despite limited data, international guidelines recommend the use of intravenous immunoglobulin (IVIg) in neonates with Rhesus hemolytic disease.

Objective

To test whether prophylactic use of IVIg reduces the need for exchange transfusions in neonates with Rhesus hemolytic disease.

Design and setting

We performed a randomized, double-blind, placebo-controlled trial in neonates with Rhesus hemolytic disease. After stratification for treatment with intrauterine transfusion, neonates were randomly assigned for IVIg (0.75 g/kg) or placebo (glucose 5%). The primary outcome was the rate of exchange transfusions. Secondary outcomes were duration of phototherapy, maximum bilirubin levels, and the need for top-up red cell transfusions.

Results

Eighty infants were included, of whom 53 (66%) were treated with intrauterine transfusion(s). There was no difference in the rate of exchange transfusions between the IVIg and placebo groups (17% (7 of 41) versus 15% (6 of 39), p = 1.00) and in number of exchange transfusions per patient (median (range): 0 (0-2) versus 0 (0-2), p = 0.90), or in duration of phototherapy (4.7 ± 1.8 versus 5.1 ± 2.1 days, p = 0.34), maximum bilirubin levels (14.8 ± 4.7 versus 14.1 ± 4.9 mg/dL, p = 0.52) and proportion of neonates requiring top-up red cell transfusions (83% (34 of 41) versus 87% (34 of 39), p = 0.76).

Conclusion

Prophylactic IVIg does not reduce the need for exchange transfusion or the rates of other adverse neonatal outcomes. Our findings do not support the use of IVIg in neonates with Rhesus hemolytic disease.

Introduction

Rhesus hemolytic disease of the neonate (HDN) may lead to excessive hyperbilirubinemia and permanent brain damage due to kernicterus. Traditional neonatal treatment of Rhesus HDN consists of intensive phototherapy and exchange transfusion (ET). Phototherapy lowers bilirubin through photo-oxidation, whereas ET removes bilirubin and hemolytic antibodies, and corrects anemia.[1] However, ET is a high-risk invasive procedure associated with a significant rate of adverse effects. Although the mortality rate associated with ET is nowadays reported to be less than 0.3% in term infants, the morbidity rates can reach 24% and includes catheter-related complications, sepsis, thrombocytopenia and hypocalcemia.[1-7] Neonatal treatment with intravenous immunoglobulin (IVIg) has been suggested as an alternative therapy for ET in Rhesus HDN.[8] In many Western countries, including the Netherlands, IVIg is widely used.[9] A few small randomized controlled trials (RCT) reported that IVIg combined with phototherapy reduces serum bilirubin levels and the need for ET in neonates with Rhesus HDN compared to phototherapy alone.[10-13] In these studies, treatment with IVIg reduced the duration of phototherapy and length of hospitalization, but increased the need for top-up red cell transfusions.

Recommendations for the routine use of IVIg are controversial due to various methodological limitations of the studies. A Cochrane review suggested in 2002 that the results of further trials of higher quality should be awaited.[14] The American Academy of Pediatrics (AAP) recommended in 2004 the use of IVIg (0.5–1 g/kg) in Rhesus HDN in case of failure of phototherapy, based on the same limited data.[8] Given these conflicting recommendations, a well-designed RCT for the use of IVIg in Rhesus HDN was urgently needed. We hypothesized that IVIg reduces the need for ET and we designed an RCT to address this question.

Patients and Methods

We performed a randomized single-center double-blind placebo-controlled trial (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=832). The Leiden University Medical Center (LUMC) is the national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. All neonates of 35 or more weeks of gestation with Rhesus HDN, born between 2006 and 2010 and admitted to the neonatal nursery of the LUMC were eligible. Rhesus HDN was defined as (1) Maternal Antibody Dependent Cellular Cytotoxicity-test (ADCC) >50%, a validated functional test predicting severe hemolysis and comparable with a titer of >1:64[15] and (2) positive direct

antiglobulin test caused by anti-Rhesus D (RhD) or c antibodies in the fetus/neonate of a RhD or c negative mother. We excluded all neonates (1) with perinatal asphyxia (defined as an Apgar score at 5 minutes less than 3 and/or umbilical cord arterial pH less than 7.0), (2) with hemolytic disease other than RhD or c and (3) with Rhesus HDN presenting >4 hours after birth.

Written informed parental consent was obtained before birth. After stratification into two groups (with and without IUT), infants were assigned at birth to the IVIg treatment group (IVIg-group) or placebo control group (placebo-group) through pharmacy-controlled randomization. Method of treatment allocation was computer-generated randomization sequence, with randomization code kept by the chief pharmacist. The block size for randomization was 4 in the IUT-group and 2 in the group without IUT (because of the expected smaller proportions of infants in the non-IUT-group). The hospital pharmacy provided identical coded drug boxes and infusion solutions were delivered in sequentially-numbered identical vials containing either IVIg or placebo. To prevent discrepancy between two children of the same family, in case of twins the same vial was used for both children. Clinicians, nurses and parents were blinded to the randomization and allocation.

In the IVIg-group, patients received conventional intensive phototherapy plus prophylactic IVIg as a single dose of 0.75 g/kg (administered in approximately 5-6 hours) starting within the first 4 hours after birth. In the placebo-group, patients received conventional intensive phototherapy plus an equal amount of glucose 5% intravenous infusion.

The IVIg product used in this trial (Nanogam®, Sanquin Amsterdam, The Netherlands) is treated with solvent-detergent to inactivate enveloped viruses and subjected to filtration through a 15 nanometer filter to remove non-enveloped viruses, including Parvo B19. Nanogam® contains more than 95% monomeric IgG and no aggregates.

All infants with Rhesus HDN admitted to our neonatal nursery receive intensive phototherapy directly after birth using white light with an intensity of 12-20 μW/cm/nm given by air shield and Ohmeda lamps, in combination with a bilirubin-blanket providing blue light 30 μW/cm/nm. During phototherapy, extra fluids (10 ml/kg) are administered. Phototherapy and ET were performed according to the latest AAP guidelines.[8] The criteria for ET were: (1) total serum bilirubin above (higher) ET thresholds and/or (2) rise of bilirubin >0.5 mg/dL/hr despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level. ET criteria were not based on fixed bilirubin thresholds, but were derived from the nomograms of AAP and varied according to postnatal age (hours/days) of the neonate. ET was performed with double-volume transfusion (160 mL/kg) using irradiated and leukocyte-depleted compatible erythrocytes.

We recorded the following obstetric and neonatal data: fetal hemoglobin (Hb) concentration and gestational age at first IUT, number of IUTs, gestational age at birth, birth weight, Hb concentration, reticulocyte count and bilirubin level from cord blood at birth, maximum bilirubin level during admission, duration of phototherapy and admission (days), number of ETs required, number of top-up red blood cell transfusions received during the first 3 months of life and Hb levels prior to top-up transfusion. Hb levels were measured routinely every week up to three months of age. After discharge from our center, top-up transfusions were performed in referring hospitals when Hb levels were <8.0 g/dL, or <9.6 g/dL in the presence of clinical symptoms of anemia (such as lethargy, feeding problems, need for oxygen or failure to thrive). Folic acid (50 mcg/day) was administered orally during the first three months of life to all neonates. Data on the number of top-up transfusions and Hb levels in infants managed (after discharge) outside our center were collected through correspondence with the local pediatrician or blood transfusion department.
Primary outcome was the rate of ET and the number of ETs per infant. Secondary outcomes were duration of phototherapy and hospital stay, maximum serum bilirubin levels and the need for top-up red cell transfusions in the first three months of life.

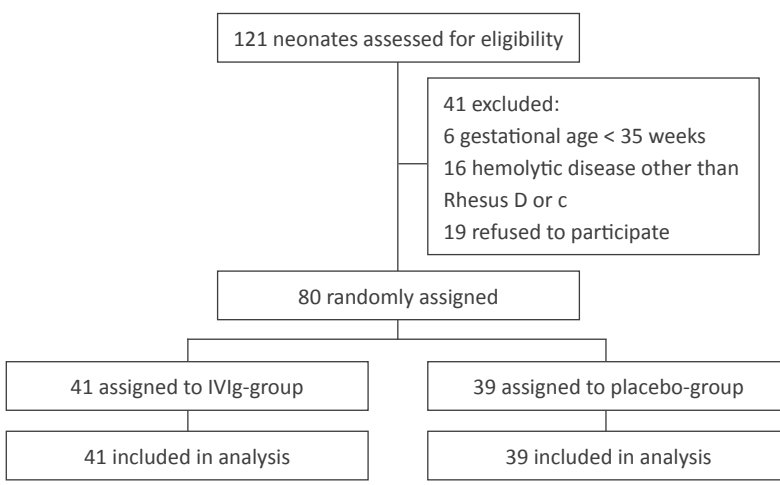
Statistical analysis

Based on the available literature, we calculated that a minimum of 40 infants in each study arm was required to demonstrate a 5-fold reduction in need for ET between the placebo-group and the IVIg-group (30% versus 6%) with a significance of 0.05 and a power of 80%, by two-tailed analysis. The expected rate (30%) of ET in the placebo-arm was derived from the recorded incidence on ET at our department in 2005-2006. The expected rate (6%) of ET in the IVIg-group was calculated from the reported data in the literature (Gottstein and Cooke)[11]. According to the meta-analysis from Gottstein and Cooke, the use of IVIg in neonates with Rhesus HDN could lead to a 5-fold reduction in the incidence of ET (relative risk (RR) 0.21, 95% confidence intervals (CI) 0.10 to 0.45).
Data are reported as means and standard deviations (SD) or as median and ranges, as appropriate. Statistical analysis was performed using Student-t-test and Mann-Whitney test for continuous variables. Chi square and Fisher's exact test were used for categorical variables, as appropriate. A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 17.0 (SPSS Inc, Chicago, IL, USA).

Results

A total of 121 neonates with Rhesus hemolytic disease were born in the study period, of whom 41 (34%) were excluded (Figure 1). We enrolled 80 patients in the study, 41 patients in the IVIg-group and 39 in the placebo-group. One pair of twins was included in the IVIg-group. Both children received IVIg from the same vial according to the protocol. During infusion of the study medication no potential side-effects such as hypotension, tachycardia or allergic reactions were reported. The baseline characteristics of the two treatment groups were similar (Table 1).

Figure 1. Flow diagram of study participants



Neonatal outcome: phototherapy and ET

All neonates were treated with intensive phototherapy directly after birth. The mean number (SD) of days of phototherapy in neonates in the IVIg-group and placebo group was 4.7 (1.8) and 5.1 (2.1), respectively (p = 0.34). At least one ET was required in 17% (7/41) of the neonates in the IVIg-group compared to 15% (6/39) in the placebo-group (p = 1.00). The median number of ETs in the IVIg-group and placebo-group was 0 (range 0-2) and 0 (range 0-2), respectively (p = 0.90). Median time from birth to (first) ET was 44 hours (range 9-60) in the IVIg-group and 31 hours (range 22-66) in the placebo-group. IVIg or placebo was administered within the first 4 hours after birth.

Table 1. Baseline characteristics of the included patients

	IVIg-group (n = 41)	placebo-group (n = 39)	p-value
Gestational age at birth, weeks[a]	36.7 ± 1.0	36.5 ± 0.6	0.23
Birth weight, grams[a]	2994 ± 485	2953 ± 424	0.68
Male, n (%)	29 (71)	25 (64)	0.64
Neonates treated with IUT, n (%)	27 (66)	26 (67)	0.99
Number of IUTs per neonate[b]	1 (0-4)	1 (0-6)	0.47
Gestational age at first IUT, weeks[a]	29 ± 4	28 ± 6	0.44
Hemoglobin level at first IUT, g/dL[a]	6.9 ± 2.2	6.5 ± 2.3	0.44
Rhesus D immunization, n (%)	36 (88)	35 (90)	0.59
Hemoglobin level at birth, g/dL[a]	12.2 ± 2.9	11.9 ± 2.6	0.52
Reticulocyte count at birth, ‰[a]	64 ± 51	52 ± 57	0.31
Bilirubin level at birth, mg/dL[a]	7.0 ± 3.9	5.7 ± 2.3	0.07

[a] Value given as mean ± SD, [b] Value given as median (range)

Maximum mean bilirubin levels during admission were similar in both groups (14.8 ± 4.7 versus 14.1 ± 4.9 mg/dL, respectively (p = 0.52). Similar results for the primary and secondary outcomes were observed for the sub-groups of neonates after stratification for treatment with or without IUT. Detailed information on neonatal treatment and outcome in both groups, overall and after stratification for IUT, is presented in Table 2.

One included patient developed a Bacillus cereus sepsis with brain abscesses a few days after an ET performed through an umbilical venous catheter. Because of this serious adverse event the randomization code for the patient was opened and showed that the infant had received IVIg. Sterility tests on the used IVIg batches were subsequently performed and found to be sterile. In addition, cultures of all donor blood products used for the IUTs and ET were examined and found to be sterile. Therefore the cause of infection remained unclear and may have been related to the umbilical venous catheterization and ET. Detailed information on this exceptional case can be found in a case report.[16]

Top-up transfusions

The percentage of neonates requiring a top-up transfusion in the IVIg-group and placebo-group was 83% (34/41) and 87% (34/39), respectively (p = 0.76). The median number of top-up transfusions per neonate in the IVIg-group and placebo-group was 2 (range 0-6) and 2 (range 0-6), respectively (p = 0.93). Mean hemoglobin level at first top-up transfusion and

Table 2. Neonatal outcomes in the IVIg-group and placebo-group and according to stratification for IUT

	Total group (n = 80)			IUT-group (n = 53)			No-IUT group (n = 27)		
	IVIg (n = 41)	**placebo** (n = 39)	**p-value**	**IVIg** (n = 27)	**placebo** (n = 26)	**p-value**	**IVIg** (n = 14)	**placebo** (n = 13)	**p-value**
Neonates with exchange transfusions, n (%)	7 (17)	6 (15)	0.99	7 (26)	4 (15)	0.50	0 (0)	2 (15)	0.22
Number of ETs per neonate[a]	0 (0-2)	0 (0-2)	0.90	0 (0-2)	0 (0-2)	0.54	0 (0-0)	0 (0-1)	0.14
Maximum bilirubin, mg/dL[b]	14.8 ± 4.7	14.1 ± 4.9	0.52	14.9 ± 5.2	12.6 ± 4.8	0.11	14.7 ± 3.8	17.0 ± 3.7	0.11
Phototherapy, days[b]	4.7 ± 1.8	5.1 ± 2.1	0.34	4.4 ± 1.6	4.5 ± 2.0	0.74	5.3 ± 1.9	6.2 ± 2.0	0.23
Hospitalization, days[b]	7 ± 4	7 ± 3	0.37	6 ± 4	7 ± 3	0.58	7 ± 4	8 ± 3	0.45

[a] Value given as median (range), [b] Value given as mean ± SD

Table 3. Top-up transfusions in neonates with Rhesus D or c hemolytic disease treated with or without IVIg

	Total group (n = 80)			IUT group (n = 53)			No-IUT group (n = 27)		
	IVIg (n = 41)	**placebo** (n = 39)	**p-value**	**IVIg** (n = 27)	**placebo** (n = 26)	**p-value**	**IVIg** (n = 14)	**placebo** (n = 13)	**p-value**
Neonates requiring top-up transfusions, n (%)	34 (83)	34 (87)	0.76	23 (85)	24 (92)	0.67	11 (78)	10 (77)	0.99
Number of top-up transfusions per neonate[a]	2 (0-6)	2 (0-6)	0.93	2 (0-6)	2 (0-6)	0.71	2 (0-5)	1 (0-5)	0.70
1 top-up transfusion, n (%)	8 (20)	10 (26)	0.51	6 (22)	5 (19)	0.79	2 (14)	5 (38)	0.21
2 top-up transfusions, n (%)	12 (29)	8 (21)	0.37	7 (26)	7 (27)	0.93	5 (36)	1 (8)	0.16
3 top-up transfusions, n (%)	6 (15)	9 (23)	0.33	4 (15)	7 (27)	0.28	2 (14)	2 (15)	0.99
4 top-up transfusions, n (%)	4 (10)	4 (10)	0.99	3 (11)	3 (12)	0.99	1 (7)	1 (8)	0.99
5 top-up transfusions, n (%)	2 (5)	2 (5)	0.99	1 (4)	1 (4)	0.99	1 (7)	1 (8)	0.99
6 top-up transfusions, n (%)	2 (5)	1 (3)	0.99	2 (7)	1 (4)	0.99	0 (0)	0 (0)	0.99
Days after birth until first top-up transfusion[b]	12 ± 12	16 ± 15	0.24	12 ± 12	16 ± 16	0.33	13 ± 11	17 ± 15	0.50
Hemoglobin level at first top-up transfusion, g/dL[b]	8.4 ± 1.3	8.1 ± 1.4	0.38	8.5 ± 1.3	8.1 ± 1.5	0.25	8.0 ± 1.1	8.1 ± 1.0	0.77

[a] Value given as median (range), [b] Value given as mean ± SD

median number of days until first top up transfusion were similar in both groups. Detailed information on the use of top-up transfusions in the IVIg-group and the placebo-group is presented in Table 3.

Discussion

In this RCT we have shown that prophylactic treatment with IVIg in neonates with Rhesus hemolytic disease did not reduce the need for ET or the rates of other adverse neonatal outcomes. Our results do not support the recommendation to give IVIg in Rhesus hemolytic disease, as stated in recent AAP guidelines[8]. Our study adds to the Cochrane analysis that there is no evidence to recommend routine use of IVIg.[14]

In the past, several studies have suggested a positive effect of IVIg in reducing the rate of hemolysis in Rhesus hemolytic disease.[10,12,13,17-19] Although the exact mechanism of action of IVIg remains unclear, IVIg has been reported to block Fc-receptors on macrophages, resulting in a decreased removal of anti-Rh antibody coated erythrocytes from the circulation. IVIg might increase IgG catabolism, resulting in a shorter half-life of antibodies (including anti-Rh antibodies). A third hypothesis is the presence of anti-idiotypic antibodies in IVIg neutralizing anti-Rh antibodies.[12,20-22]

Our results are in contrast with the most recent recommendations of the AAP to use 0.5-1.0 g/kg IVIg in Rhesus hemolytic disease in case of failure of phototherapy.[8] These guidelines were published in 2004 and based on a limited number of small RCTs. Several important methodological limitations hampered the interpretation of these studies, including sub-optimal study-designs and the wide range of inclusion criteria.[18,19] The Cochrane Collaboration performed a review on three studies, in which a total of 189 infants were included.[10,12-14] Rubo et al.[10] included 32 infants with Rhesus hemolytic disease in a multicenter RCT. No details on IUT and gestational age were given. Several years later Dagoglu et al.[13] included 29 preterm and 12 term infants in an RCT. Cut-off for prematurity and criteria for top-up red cell transfusions were not defined. In 1999 Alpay et al.[12] enrolled 116 infants, predominantly with ABO incompatibility (n = 93), but also neonates with Rhesus hemolytic disease (n = 16) and both Rh and ABO incompatibility (n = 7) were included. However, results were not given for each group separately. None of the studies described detailed phototherapy guidelines and none of them used a placebo in the control-group or described any method of blinding the intervention after allocation concealment. According to the Cochrane review, none of the trials fulfilled criteria for high quality study. Our study is the first well-designed randomized double-blind placebo-controlled trial on this topic.

In 4 other studies, infants with ABO incompatibility were included.[12,17,18,23] In general, compared to Rhesus immunization, ABO incompatibility causes less severe hemolysis and therefore less neonatal morbidity.[24] For that reason, we included only neonates with Rhesus disease. These important methodological differences between our study and the previous ones may explain the discordant results.

Several other explanations can be envisaged to explain the lack of effect of IVIg in our study. A possible explanation could be the treatment with intensive and prophylactic phototherapy starting immediately after birth, thereby reducing the risk of severe hyperbilirubinemia. In addition, the majority of infants included in our study were treated with IUT. By IUT, Rhesus incompatible erythrocytes of the fetus are replaced by Rhesus compatible cells of the donor. Dependent on the interval between the last IUT and delivery, these donor cells are still present after birth, resulting in less or more delayed hemolysis.[25, 26] However, several groups including ours have shown that even after IUT, neonates with Rhesus hemolytic disease still often require ET.[27,28] In our study, IVIg was neither effective in the IUT group or in the group without IUT. However, the number of patients included in the subgroup without IUT (n = 27) may be too small to draw firm conclusions. Recently, a research group from Brazil finalized a similar RCT on IVIg for neonates with Rhesus hemolytic disease and, in accordance with our results, found no difference between both groups on the rate of ET. Importantly, in their study the vast majority of patients (n = 80) had no prior treatment with IUT (ClinicalTrials.gov NCT00288600).[29] Therefore, both our RCT and the RCT from the Brazilian research group failed to show any effect of IVIg in Rhesus hemolytic disease infants, irrespective of whether or not the infants were treated with IUT. Care should be taken when interpreting our results, particularly the sub-group analyses, due to the relatively limited number of patients. In addition, caution should be used before applying the results of this study to all Rhesus isoimmunized infants. There may be a subset of Rhesus isoimmunized infants with (inappropriate) delayed start of intensive phototherapy, for whom IVIg might be effective. More studies are needed to study the effect of IVIg in this specific subset of infants.

Although IVIg is considered to be an extremely safe product, adverse events can not be totally eliminated. Rare but serious side effects such as transfusion transmitted diseases, anaphylaxis, hypersensitivity, thrombosis, pulmonary emboli and renal failure have been reported.[21,22] Recently, Figueras-Aloy et al.[30] reported a higher incidence of necrotizing enterocolitis (NEC) in near-term infants with Rhesus hemolytic disease treated with IVIg compared to a control group managed without IVIg. The authors correctly suggest that their results must be interpreted with care given the retrospective nature of the study. Whether

occurrence of NEC was related to the administration of IVIg or to the fact that infants receiving IVIg were more ill than the control group is not clear. Nevertheless, since potential (but rare) adverse effects associated with the use of IVIg can not be ruled out, the authors call for more caution when using IVIg in neonates with Rhesus hemolytic disease. IVIg is a blood product prepared by separating the gamma-globulin fraction from the plasma pooled from multiple donors. The manufacturing of IVIg, including fractionation and filtration of viruses is an extremely intensive and expensive process. Therefore, the use of IVIg for indications that are not confirmed by well-designed RCTs should be restricted.[22]
In conclusion, prophylactic treatment with IVIg (in a dosage of 0.75 g/kg) did not reduce the need for ET or the rates of other adverse neonatal outcomes. Our findings do not support the current recommendations of the AAP to use IVIg in neonates with Rhesus hemolytic disease. In view of the absence of beneficial effects, the use of IVIg for this indication should be discouraged.

Acknowledgement

Nanogam® was provided by Sanquin Blood Supply Foundation (Amsterdam, The Netherlands).

References

1. 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.
2. Keenan WJ, Novak KK, Sutherland JM, Bryla DA, Fetterly KL. Morbidity and mortality associated with exchange transfusion. Pediatrics. 1985;75:417-421.
3. 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.
4. Jackson JC. Adverse events associated with exchange transfusion in healthy and ill newborns. Pediatrics. 1997;99:E7.
5. 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.
6. Thayyil S, Milligan DW. Single versus double volume exchange transfusion in jaundiced newborn infants. Cochrane Database Syst Rev. 2006;CD004592.
7. Hovi L, Siimes MA. Exchange transfusion with fresh heparinized blood is a safe procedure. Experiences from 1 069 newborns. Acta Paediatr Scand. 1985;74:360-365.
8. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114:297-316.
9. New HV, Stanworth SJ, Engelfriet CP et al. Neonatal transfusions. Vox Sang. 2009;96:62-85.
10. Rubo J, Albrecht K, Lasch P et al. High-dose intravenous immune globulin therapy for hyperbilirubinemia caused by Rh hemolytic disease. J Pediatr. 1992;121:93-97.
11. 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.
12. Alpay F, Sarici SU, Okutan V, Erdem G, Ozcan O, Gokcay E. High-dose intravenous immunoglobulin therapy in neonatal immune haemolytic jaundice. Acta Paediatr. 1999;88:216-219.
13. Dagoglu T, Ovali F, Samanci N, Bengisu E. High-dose intravenous immunoglobulin therapy for rhesus haemolytic disease. J Int Med Res. 1995;23:264-271.
14. Alcock GS, Liley H. Immunoglobulin infusion for isoimmune haemolytic jaundice in neonates. Cochrane Database Syst Rev. 2002;CD003313.
15. 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;184:1015-1020.
16. Smits-Wintjens VE, Steggerda SJ, Oepkes D, Van Kamp IL, Kramer CM, Walther FJ et al. Bacillus cereus cerebral abscesses in a term neonate with rhesus hemolytic disease treated with exchange transfusion. J Pediatr Inf Dis. 2010;5:277-280.
17. Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. Saudi Med J. 2006;27:1827-1830.
18. Tanyer G, Siklar Z, Dallar Y, Yildirmak Y, Tiras U. Multiple dose IVIG treatment in neonatal immune hemolytic jaundice. J Trop Pediatr. 2001;47:50-53.
19. Voto LS, Sexer H, Ferreiro G et al. Neonatal administration of high-dose intravenous immunoglobulin in rhesus hemolytic disease. J Perinat Med. 1995;23:443-451.

20. Kriplani A, Malhotra SB, Mandal K. Fetal intravenous immunoglobulin therapy in rhesus hemolytic disease. Gynecol Obstet Invest. 2007;63:176-180.
21. 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.
22. Kumar A, Teuber SS, Gershwin ME. Intravenous immunoglobulin: striving for appropriate use. Int Arch Allergy Immunol. 2006;140:185-198.
23. Miqdad AM, Abdelbasit OB, Shaheed MM, Seidahmed MZ, Abomelha AM, Arcala OP. Intravenous immunoglobulin G (IVIG) therapy for significant hyperbilirubinemia in ABO hemolytic disease of the newborn. J Matern Fetal Neonatal Med. 2004;16:163-166.
24. Murray NA, Roberts IA. Haemolytic disease of the newborn. Arch Dis Child Fetal Neonatal Ed. 2007;92:F83-F88
25. Oepkes D, Adama van SP. Intrauterine fetal transfusions in the management of fetal anemia and fetal thrombocytopenia. Semin Fetal Neonatal Med. 2007;12:432-438.
26. 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;104:818-824.
27. 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.
28. Rath ME, Smits-Wintjens VE, Lindenburg I et al. Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang. 2010;99(1):65-70.
29. Santos MC, Sa, Gomes, Camacho, Moreira. High-dose intravenous immunoglobulin therapy for hyperbilirubinemia due Rh hemolytic disease: a randomized clinical trial. Pediatric Academic Societies-annual meeting-Vancouver 2010. 2010;143.
30. 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.

Chapter 6

Cochrane systematic review: Immunoglobulin for alloimmune disease in neonates

Mirjam E.A. Rath
Vivianne E.H.J. Smits-Wintjens
Enrico Lopriore
Helen Liley

Submitted

Abstract

Background

Exchange transfusion (ET) and phototherapy have traditionally been used to treat jaundice and avoid the associated neurological complications. Because of the risks and burdens of ET, intravenous immunoglobulin (IVIg) has been suggested as an alternative therapy for alloimmune hemolytic disease of the newborn (HDN) to reduce the need for ET.

Objectives

To assess whether, in newborns with alloimmune HDN, IVIg is effective in reducing the need for ET.

Search methods

Electronic searches were made of PubMed, Embase (OVID version), COCHRANE Library (including CENTRAL), Web of Science, CINAHL (EbscoHost-version), Academic Search Premier and the trial registers clinicaltrials.gov and controlled-trials.com. Reference lists of included and excluded trials and relevant reviews were searched for further relevant studies.

Selection criteria

All randomized and quasi-randomized controlled trials of the use of IVIg in the treatment of alloimmune HDN were considered. Trials must have used predefined criteria for both IVIg and ET therapy to be included.

Data collection and analysis

The standard methods of the Cochrane Collaboration and its Neonatal Review Group were used. Studies were assessed for inclusion and quality and data were extracted by two reviewers working independently. Any differences of opinion were discussed and a consensus reached. Investigators were contacted for additional or missing information. For categorical outcomes, relative risk (RR) and number needed to treat (NNT) were calculated. For continuous variables, mean difference (MD) was calculated.

Results

Fourteen studies fulfilled inclusion criteria and included a total of 942 infants. Term and preterm infants with Rh and/or ABO incompatibility were included. The use of ET decreased significantly in the immunoglobulin treated group (RR 0.40, 95% CI 0.27 to 0.59; NNT 5.4).

Mean number of ETs per infant was also significantly lower in the immunoglobulin treated group (MD -0.31, 95% CI -0.45 to -0.17). However, subgroup analysis of the only two high quality studies showed no difference in the need for or number of ETs. Two studies assessed long-term outcomes and found no cases of kernicterus, deafness or cerebral palsy.

Authors' conclusions

Although overall results show a significant reduction in the need for ET in infants treated with IVIg, the applicability of the results is limited. The only two high quality studies show no benefit of IVIg in reducing the need for and number of ET. Further well designed studies are needed before solid advice can be given about the use of IVIg for the treatment of alloimmune HDN.

Background

Description of the condition

The use of anti-D prophylaxis in Rh D negative women has led to a marked decline in Rh hemolytic disease of the newborn (HDN). However, anti-D immunoglobulin is in short supply world-wide. Sensitization can occur despite immunoprophylaxis, particularly if it is given too late or in insufficient dose. Fetal therapy has led to a reduction in severity of disease in Rh sensitized fetuses, but it does not comprehensively prevent need for neonatal treatment. A proportion of significant HDN is caused by antibodies to antigens other than Rh D and is therefore not preventable with anti-D immunoglobulin. Primary modes of postnatal therapy include phototherapy and exchange transfusion (ET) to reduce risk of mortality and kernicterus. Top-up transfusions are used to treat early and late anemia. In contemporary perinatal centers, 15-40% of neonates admitted for Rh or ABO HDN require at least one ET.[1,2]

The safety of ET has been reported for over 50 years. Published mortality rates vary from 0.53-4.7% per infant.[3-9] ET-related death is more common in sick or premature infants than in healthy term infants.[1,4,6,7] Rates of morbidity and ET-related adverse events as high as 74% have been reported. Risks related to ET include adverse cardio-respiratory events, catheter-related complications, those related to the use of blood products, metabolic derangements and other serious complications such as pulmonary hemorrhage, necrotizing enterocolitis, and bowel perforation.[1,3,6,7,9-12] Because improved perinatal care has reduced the need for ET, the complication rate could increase as clinicians become less experienced with the procedure.[1] However, Steiner et al. reported that over a 21 year period, despite a sharp decline in the number of ETs performed, no increase in morbidity and mortality was observed.[1]

Description of the intervention

Intravenous immunoglobulin (IVIg) is an alternative therapy that may be effective in treating alloimmune HDN. In 1987 the first report of successful treatment of late anemia due to Rh E incompatibility with IVIg was published.[13] Subsequent case reports and case series reported success of IVIg treatment in neonates with both Rh or ABO incompatibility.[14-16] However, Hammerman et al. found a reduced or no response to IVIg treatment in infants with ABO incompatibility who had early and severe hemolysis.[17] In the last two decades several quasi-randomized or randomized controlled trials on the use of IVIg to reduce ET have been published. Timing of administration of IVIg varied from a few hours to several days after birth, single doses varied from 0.5 g/kg to 1 g/kg and total doses from 0.5 g/kg to 2.25 g/kg.

The potential benefits of IVIg over ET include that the treatment is less complicated and less labor intensive. In addition, IVIg could allow safe treatment of some infants in less sophisticated neonatal units, or avoid delaying treatment whilst transferring infants for ET. Comprehensive assessment of IVIg in premature infants, particularly in the treatment of sepsis, has shown that it is safe and well tolerated.[18] It is a well-established therapy for alloimmune thrombocytopenia due to maternal and fetal human platelet antigen (HPA) incompatibility. The risk of transmission of viral infection is extremely low.[19] Hemolysis and acute renal failure have been reported as uncommon complications of IVIg treatment.[20] One study showed an increased incidence of sepsis in premature infants receiving prophylactic IVIg.[21] A recent case-control study showed a higher incidence of necrotizing enterocolitis in near-term infants with Rh HDN treated with IVIg.[22] Other rare serious side effects of IVIg have been described in pediatric and adult cohorts, but not in newborns.[23]

How the intervention might work

IVIg might reduce the rate of hemolysis in alloimmune HDN by non-specific blockade of Fc-receptors. Red blood cells are probably destroyed by an antibody-dependent cytotoxic mechanism mediated by Fc-receptor bearing cells of the neonatal reticuloendothelial system.[24] Ergaz et al. demonstrated a decline in carboxyhemoglobin levels in four of five infants treated with IVIg for alloimmune HDN.[25] Hammerman et al. demonstrated a significant reduction in carboxyhemoglobin levels in 19 of 26 Coombs positive infants treated with IVIg.[26] Carboxyhemoglobin levels are a sensitive index of hemolysis and hence these studies suggest that immunoglobulin could decrease hemolysis.

Why it is important to do this review

This is an update of a Cochrane review first published in 2002. Although results of the previous review showed a significant reduction in the need for ET in infants treated with IVIg, the applicability of the results was limited because none of three included studies was of high quality. Nevertheless, American Academy of Pediatrics (AAP) guidelines recommend the administration of 0.5-1 g/kg IVIg in alloimmune HDN if total serum bilirubin (TSB) is rising despite intensive phototherapy or if TSB level is within 34-51 µmol/L (2-3 mg/dL) of exchange level.[27] As a result of these guidelines, despite the equivocal conclusions of the previous Cochrane review, the use of IVIg in alloimmune HDN has become widespread in many countries. However supplies of IVIg are limited and it does present some hazards. Therefore, use of IVIg should be restricted to treatment of conditions for which it is of proven benefit.

Objectives

To assess the effect of IVIg in newborn infants with alloimmune HDN on the need for and number of ETs. The review also assesses complications of therapy, short-term outcomes such as bilirubin levels, duration of phototherapy and hospitalization, top-up transfusion requirements, and long-term outcomes such as hearing loss, kernicterus and cerebral palsy.

Methods

Criteria for considering studies for this review

Types of studies

All randomized and quasi-randomized controlled trials of IVIg in the treatment of alloimmune HDN.

Types of participants

Neonates with alloimmune HDN due to either Rh or ABO blood group antibodies with or without any other blood group antibodies.

Types of interventions

IVIg given for treatment of alloimmune HDN, versus control (placebo or "standard care"). Early and late IVIg administration have been defined (for this review) as IVIg started within or after the first 12 hours of life, respectively. Studies should include predefined criteria for both IVIg and ET therapy.

Types of outcome measures

Primary outcomes

Efficacy:

- Use of ET (proportion of infants receiving one or more ETs)
- ETs performed per infant

Secondary outcomes

Efficacy:

- Use of top-up transfusion(s) in first week of life (% of infants)
- Number of top-up transfusions performed in first week of life per infant
- Use of top-up transfusion(s) after first week of life (% of infants)
- Number of top-up transfusions performed after first week of life per infant
- Maximum total serum bilirubin (TSB) (µmol/L (mg/dL))

- Duration of phototherapy (days)
- Duration of hospitalization (days)
- Incidence of sensorineural hearing loss (any severity)
- Incidence of kernicterus
- Incidence of cerebral palsy

Safety:

- Neonatal mortality
- Incidence of adverse reactions possibly related to the use of IVIg or ET

Search methods for identification of studies

Electronic searches

We performed a search in PubMed, Embase (OVID version), COCHRANE Library (including CENTRAL), Web of Science, CINAHL (EbscoHost-version), and Academic Search Premier. The subject query was applied in all databases taking into account the terminological differences between these databases. The query consisted of the combination of four subjects: immunoglobulins, alloimmune hemolytic jaundice, newborn infants, and randomized controlled trials. Various synonyms and related terms for all subjects were used. Two search strategies were used: the first strategy was limited to randomized trials and systematic reviews, the second strategy included only the subjects immunoglobulins and alloimmune hemolytic disease (and synonyms and related terms for those subjects). The search was performed on the 22nd of March 2012. The bibliographic databases yielded 1251 references in total of which titles and abstracts were screened. In addition to database searches, searches were made of the trial registers clinicaltrials.gov and controlled-trials.com. No language restrictions were applied.

Searching other resources

We searched the reference lists of all included and excluded trials and relevant reviews for further relevant studies.

Data collection and analysis

The standard method of the Cochrane Collaboration and its Neonatal Review Group was used.

Selection of studies

Two reviewers independently screened all 1251 references for possible inclusion using predefined criteria for inclusion (see below). If a report appeared to meet inclusion criteria

for the review, or if it was not clear based on title and abstract, a full text version of the article was obtained. Any disagreements were resolved through discussion.
The inclusion criteria for this review were:

- Randomized and quasi-randomized controlled trials
- Study compared IVIg with any definition of "standard care" plus placebo, or with any definition of "standard care" without placebo
- Study included patients with alloimmune HDN due to either ABO or Rh blood group antibodies with or without any other blood group antibodies
- Study measured ETs (primary outcome) for each study arm and/or at least one of the secondary outcomes for each study arm
- Study used predefined criteria for both IVIg and ET therapy

Data extraction and management
Two review authors independently extracted data using a data collection form that was pilot tested before use. Any disagreements were resolved through discussion and if necessary with the help of a third reviewer blinded to trial author, institution and journal of publication. One review author contacted authors of studies that did not report all required data or information. Data were entered into Review Manager 5.1 by one review author and checked by at least one review author.

Assessment of risk of bias in included studies
Two review authors independently assessed the risk of bias in included studies using the 'Risk of bias' tool as described in the Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0 (Higgins 2011).[28] The following items for risk of bias were assessed: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Each item was rated as 'Low risk of bias', 'Unclear risk of bias' or 'High risk of bias'. Any differences of opinion were discussed with a third blinded reviewer and a consensus reached. For selective reporting the following criteria were used to rate a study as 'Low risk of bias':

- For studies enrolling neonates with Rh or both Rh and ABO HDN: reporting (in paper or subsequent correspondence) at least one outcome related to each of ET, bilirubin and top-up transfusion, plus adverse effects and hospitalization.
- For studies enrolling only neonates with ABO HDN: reporting (in paper or subsequent correspondence) at least one outcome related to each of ET and bilirubin, plus adverse effects and hospitalization. Top-up transfusion was not considered to be a preferred

outcome measure because anemia requiring treatment is an unusual consequence of ABO alloimmune hemolysis.

- Study protocols or methods section of papers should not describe an intention to report outcomes that were not subsequently reported in the paper.

Measures of treatment effect

For categorical outcomes, such as the incidence of ET, the relative risk (RR) was calculated. For continuous variables, such as the maximum bilirubin level, the mean difference (MD) was calculated. The number needed to treat (NNT) to avoid ET was also calculated.

Dealing with missing data

Investigators were contacted for missing information about study design and/or results.

Assessment of heterogeneity

Clinical heterogeneity was assessed by determining whether clinical characteristics of patients, interventions, outcome measures and timing of outcome measurements were similar for included studies. Statistical heterogeneity was assessed using Chi^2 and I^2 tests. An I^2 test of ≥50% was considered as substantial or considerable heterogeneity according to the Cochrane Handbook.[28]

Assessment of reporting biases

Funnel plots were used to assess publication bias for those outcomes with ≥10 trials. No substantial asymmetry was encountered in the funnel plots.

When selective reporting bias was suspected based on the criteria described under 'Assessment of risk of bias in included studies', investigators were contacted to request the missing outcome data.

If the data remained unavailable and the absence was thought to introduce serious bias, the impact of including such studies was explored in the overall assessment of results by a sensitivity analysis.

Data synthesis

Review Manager 5.1 was used to synthesize the available data. Whether a fixed-effect model or a random-effects model was used, depended on the level of clinical heterogeneity, the results of the Chi^2 test and I^2 statistic for heterogeneity[28] and the number of included studies for an outcome. If substantial heterogeneity was detected and the number of included studies on that outcome was ≥10, a random-effects model was used and the

sources of heterogeneity examined. If no substantial statistical heterogeneity was detected or the number of included studies on an outcome was <10, a fixed-effect model was used.

Subgroup analysis and investigation of heterogeneity
Subgroup analyses were conducted to determine if effects depend on:
Population:
1. Rh incompatibility
2. ABO incompatibility
3. Gestational age at birth (<37 weeks and ≥37 weeks)

Intervention:
1. Early administration of IVIg: start of IVIg ≤12 hours after birth
2. Late administration of IVIg: start of IVIg >12 hours after birth
3. Single versus multiple doses

Quality of studies (see 'Sensitivity analysis')

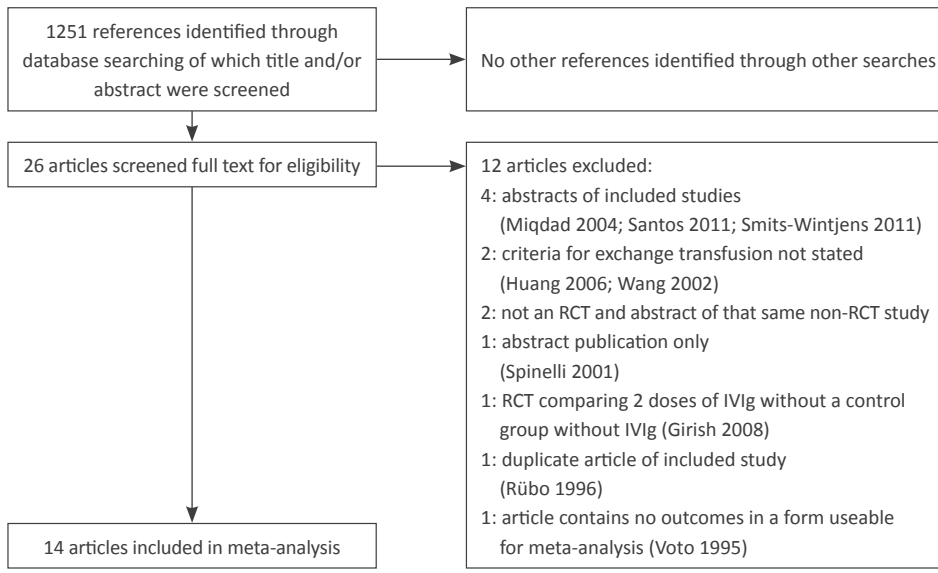
Sensitivity analysis
We conducted a sensitivity analysis based on the quality of included studies. We considered a study to be of high quality if it was rated as low risk of bias for random sequence generation, allocation concealment, blinding (performance and detection bias), incomplete outcome data, selective reporting and other risk of bias (if present).

Results

Description of studies
Detailed information of included and excluded studies is provided in Appendix 1 and 2.

Results of the search
The search conducted up until 22 March 2012 identified a total of 1251 references. After title and abstract screening, the full text of 26 references was screened. After full text screening 14 studies were included in the meta-analysis (Rübo 1992; Dağoğlu 1995; Atici 1996; Rübo 1996; Alpay 1999; Pishva 2000; Tanyer 2001; Garcia 2004; Miqdad 2004; Nasseri 2006; Elalfy 2011; Hematyar 2011; Smits-Wintjens 2011; Santos 2011).[2,29-41] Details of the studies are given in the table of included studies. Two studies have been excluded until further information is available from the authors.[42,43] Three studies have been permanently excluded from this review. Details of these studies are given in appendix 2. Searching reference lists of included and excluded studies and relevant reviews has not resulted in additional studies. A flow diagram of the study selection process is presented in Figure 1.

Figure 1. Flow diagram of the study selection process



Included studies

Fourteen randomized controlled trials published between 1992 and 2011 were included in this review.

Participants

The 14 included studies comprised 942 participants. Six studies included only infants with Rh incompatibility.[2,31-33,37,40] One study included only infants with ABO incompatibility.[35] Four studies enrolled mostly infants with ABO incompatibility but also some with Rh incompatibility and both ABO and Rh incompatibility (Alpay 1999: 93 ABO, 16 Rh, 7 both; Atici 1996: 49 ABO, 23 Rh; Hematyar 2011: 62 ABO, 11 Rh, 7 both; Nasseri 2006: 21 ABO, 13 Rh).[29,30,34,36] Pishva et al. predominantly included infants with Rh incompatibility (n = 37) and only three infants with ABO incompatibility.[38] Rübo et al. (Rübo 1996) stated that they included infants with Rh, Kell and Duffy incompatibility, although the breakdown into these groups is unclear from the paper.[39] Tanyer et al. included 34 infants with ABO incompatibility, 18 with Rh incompatibility, 2 with "subgroup" incompatibility and 7 with "more than one incompatibilities".[41] Only Nasseri et al. reported results for each type of incompatibility separately and Alpay et al. provided this information through correspondence.[29,36] Five studies enrolled only term infants ≥37 weeks of gestation.[29,30,32,36,41] None of the studies only included premature infants <37 weeks of gestation. Three studies did not describe details of the gestational age at birth of enrolled infants.[38-40] Santos et al. and Smits-Wintjens et al. provided outcomes for term and preterm infants separately.[2,37]

Interventions

Nine of 14 studies which met inclusion criteria examined the effect of a single dose of IVIg in combination with phototherapy.[2,29-32,35,37,38,40] Two studies examined multiple doses[33,36] and two studies compared groups treated with a single dose or multiple doses with a control group.[39,41] Tanyer et al. were inconsistent in describing which group received a single dose or multiple doses of IVIg and therefore this study was excluded from the subgroup analysis of single and multiple doses.[41] In the study of Hematyar et al. 15 infants received a single dose of IVIg, also 15 infants received 2 doses and 9 infants received 3 doses of IVIg.[34] Results were not provided separately for subgroups treated with a single dose and multiple doses of IVIg. Three studies used a placebo in addition to phototherapy for the control groups.[2,33,37] The intensity and topography of phototherapy fits the definition of intensive phototherapy in only three studies.[2,32,37] Tanyer et al. used an obsolete model with 3 overhead lights from a single angle and Miqdad et al. did not use a phototherapy blanket beneath the baby.[35,41] The remainder of included studies did not describe the intensity and topography of phototherapy in sufficient detail to allow a conclusion as to whether it is reasonable to describe it as intensive phototherapy. Seven studies started IVIg ≤12 hours after birth[2,31-33,37,39,40] and five studies >12 hours.[29,30,34,36,41] Miqdad et al. started IVIg within 12 hours in 9 patients and >12 hours in 47 patients, but they did not report outcomes for early and late IVIg administration separately.[35]

Outcomes

All included studies reported ET as the primary outcome. In the abstract of Garcia et al. the number of ETs per infant was reported and data on the number of infants who received one or more ETs were provided by the authors.[33] For nine studies mean (or median) number of ETs per infant were reported[2,33,36,38] or could be calculated from reported data.[30-32,40,41] Unpublished data (standard deviation and/or mean) on this outcome were provided by authors of five studies.[2,29,33,35,37] The maximum bilirubin level was reported in five studies.[2,31,37,39,40] Unpublished data on this outcome were provided by the authors of four studies.[29,32-34] Although all studies commented on the duration of phototherapy in their results, the numerical data were reported or subsequently provided in ten studies.[2,29,30,32-37,41] These studies, except for Hematyar et al., all used predefined criteria for commencing phototherapy but not all for ceasing it. Eight studies reported or subsequently provided numerical data on the duration of hospitalization.[2,29,30,32,34-37] and Pishva et al. reported only a comment.[38] Only three studies reported (after correspondence) predefined criteria for hospital discharge.[34,35,37] Seven studies included top-up transfusion as an outcome.[2,29,31,35,36,39,40] Additional data on top-up transfusions were provided by authors of

Garcia 2004; Elalfy 2011; Smits-Wintjens 2011 and Santos 2011.[2,32,33,37] Smits-Wintjens et al. did not report top-up transfusions separately for the first week and after the first week of life, but subsequently provided this information.[2] Elalfy et al. and Garcia et al. had a follow up period of only one week after discharge and until discharge, respectively.[32,33] Predefined criteria for top-up transfusions were reported in only three studies[2,29,36] and were later provided through correspondence by Santos et al.[37] All studies reported on short-term adverse events and Garcia et al.[33] provided additional information after correspondence. None of the included studies reported data on neurodevelopmental outcomes. Additional information on neurodevelopmental outcomes was provided by Miqdad et al. and Santos et al.[35,37]

Excluded studies

In total, six studies were excluded after review by authors. One study had a retrospective design, one study only compared groups with a high or a low dose of IVIg[44], and one study was only reported in abstract form.[45] Two studies did not report pre-defined criteria for the primary outcome ET.[42,43] One study did not report any outcome in a form usable for meta-analysis.[46] Details of excluded studies are given in Appendix 2.

Additional data

We have attempted to contact the authors of all studies (except for the seven studies which were identified for the previous review[29,31,39-41,45,46]) to request both further methodological information and results. We have to date successfully contacted the authors of ten papers (including contact for the previous review).[2,29,32-35,37,39,40,42]

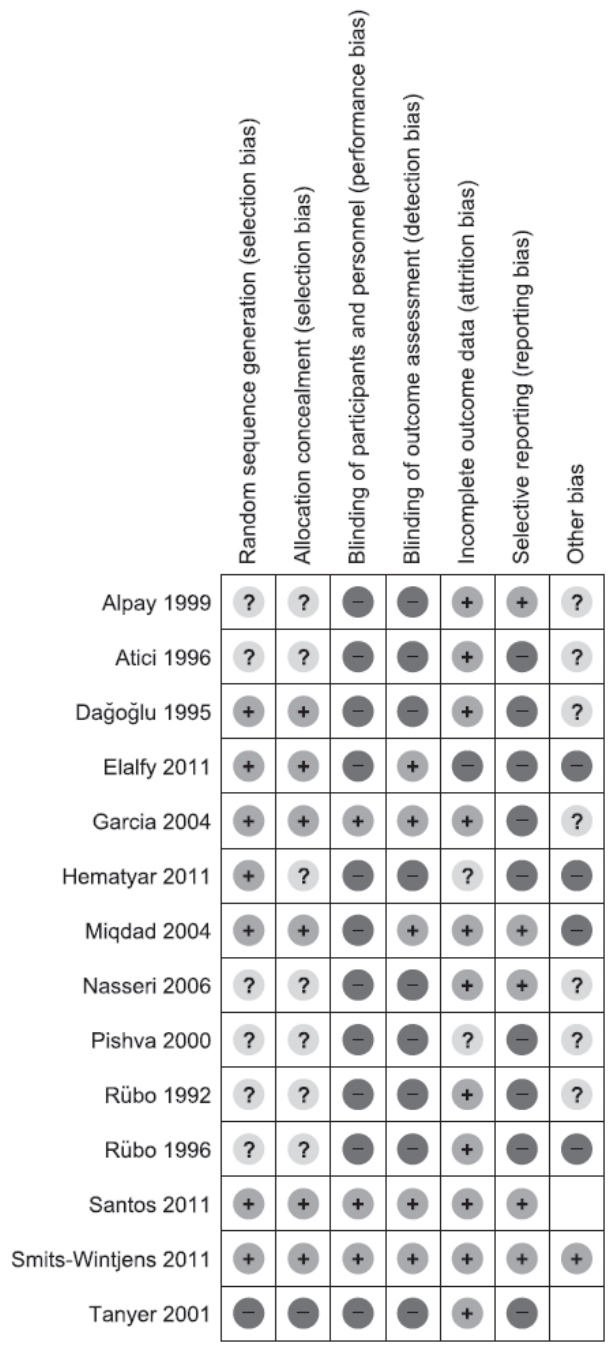
Risk of bias in included studies

For details of risk of bias of included studies, see Appendix 1 and Figure 2.

Allocation (selection bias)

Only seven studies reported an adequate method of randomization and were rated as low risk of bias.[2,31-35,37] Garcia et al., Miqdad et al., Elalfy et al., and Hematyar et al. provided information on randomization method only through correspondence.[32-35] A quasi-RCT allocated participants by order of admission.[41] This study was rated as high risk of bias for both random sequence generation and allocation concealment.

Figure 2. Summary of risk of bias of included studies



Blinding (performance bias and detection bias)

Only three studies used a placebo in the control group and were rated as low risk of bias for performance bias and detection bias.[2,33,37] Miqdad et al. reported through subsequent correspondence that data were kept and entered to their database by personnel who were not involved in the management of the cases and this study was therefore rated as low risk of detection bias.[35] Elalfy et al. replied to correspondence that the person who performed the randomization was different from the one who conducted the study and the one who analyzed the data, so their study was also rated as low risk of detection bias.[32] None of the other studies described any method of blinding of intervention after allocation and were rated as high risk of bias on both items.

Incomplete outcome data (attrition bias)

Reporting of outcome data was rated as low risk of bias in 11 studies.[2,29-31,33,35-37,39-41] For nine of these studies there were no missing data. In the Rübo 1992 trial, the amount of and reasons for missing data were similar between groups and in the study by Garcia et al. the missing data related only to 3 patients who were excluded after randomization because their blood type was Rh negative.[33,40] One study was rated as high risk of bias because of a substantial amount of missing data on bilirubin levels.[32]

Selective reporting (reporting bias)

Reporting bias was suspected in nine studies because important outcomes were either not reported or were not reported in a form that was useable for meta-analysis, or that allowed judgement about local treatment practices (for example if the authors only stated that there was no significant difference between groups).[30-34,38-41]

Other potential sources of bias

Two studies had non-random crossover after randomization,[32,39] one study used an additional criterion for ET in the control group only[35] and in two studies analysis was not performed on an intention to treat basis.[34,39] These four studies were rated as high risk of bias. Dağoğlu et al. used post randomization consent and although follow-up was complete for all infants for whom consent was obtained, two infants randomized to each arm of the study were excluded because consent was withheld.[31] Two infants were also excluded post-randomization in the Rübo 1992 study because of “protocol violations” but no details were given.[40] The latter two studies were rated as unclear risk of bias because the review authors were unable to assess the impact of these withdrawals on overall outcomes. Six other studies were rated as unclear risk of bias or low risk of bias for a potential risk of bias.[2,29,30,33,36,38] For details see “Risk of bias tables” in Appendix 1.

Effects of interventions

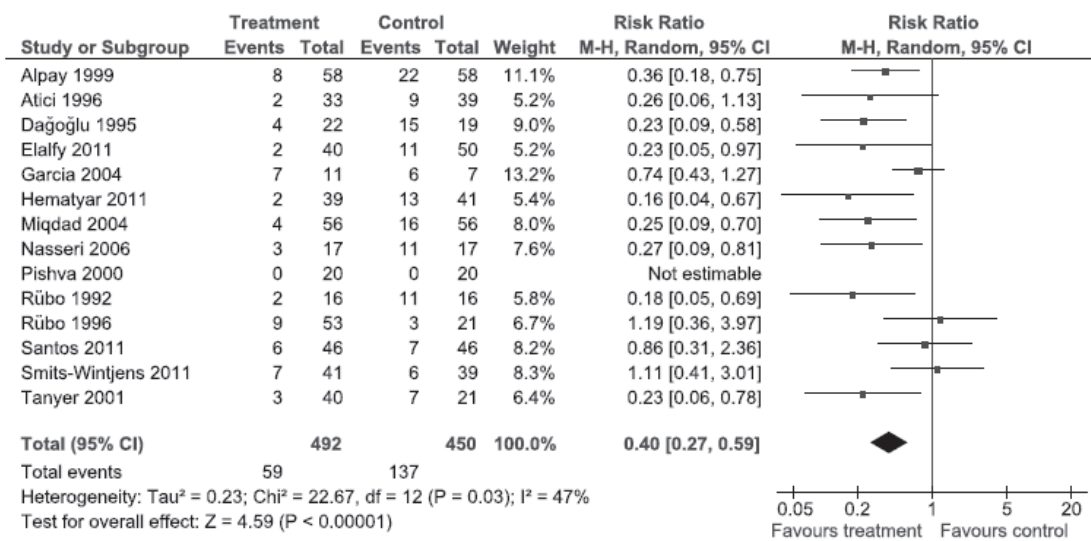
A complete overview of the results of all analyses is provided in Appendix 3.

Primary outcomes

Exchange transfusion

The results of 14 included studies could be entered into the meta-analysis.[2,29-41] Most studies found a statistically significant reduction in the use of ET for IVIg treated infants.[29-32,34-36,40,41] Four studies concluded that the use of (one or more) ETs was not reduced despite using early IVIg in combination with phototherapy.[2,33,37,39] The meta-analysis of all fourteen studies showed that IVIg reduces the need for an ET (RR 0.40, 95% CI 0.27 to 0.59; NNT 5.4) (see Figure 3).

Figure 3. Forest plot of the use of exchange transfusion (one or more)

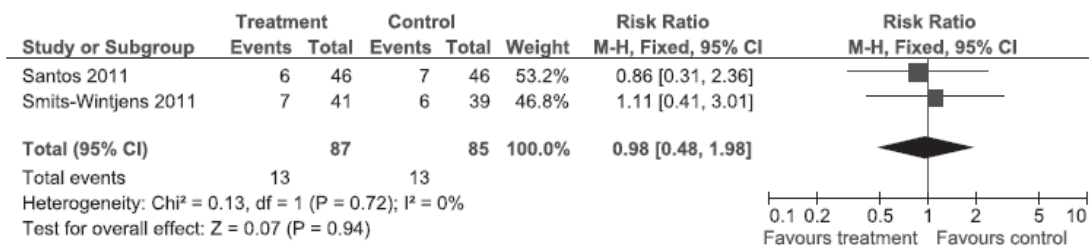


Subgroup analysis of infants with only Rh incompatibility supports a reduction in the use of ET with IVIg treatment (RR 0.42, 95% CI 0.29 to 0.61; NNT 5.7).[2,29,31-33,36-38,40] Analysis of infants with only ABO incompatibility also showed a reduction in the use of ET (RR 0.33, 95% CI 0.18 to 0.60; NNT 4.7).[29,35,36,38] In only those infants born ≥37 weeks of gestation IVIg reduced the use of ETs (RR 0.37, 95% CI 0.24 to 0.57; NNT 5.7).[2,29,30,32,36,37,41] In the subgroup of infants born <37 weeks of gestation IVIg did not reduce the use of ETs (RR 0.77, 95% CI 0.31 to 1.91).[2,37] Reductions in the use of ET were also found in the seven studies in which IVIg was used ≤12 hours after birth[2,31-33,37,39,40] (RR 0.51, 95% CI 0.35 to 0.73; NNT 7.3) and

in the five studies which used IVIg >12 hours after birth[29,30,34,36,41] (RR 0.27, 95% CI 0.17 to 0.44; NNT 3.9). Subgroup analyses of both infants receiving a single dose of IVIg [2,29-32,35,37-40] and those receiving multiples doses of IVIg[33,36,39] support a reduction in the use of ET with IVIg treatment (RR 0.41, 95% CI 0.29, 0.57; NNT 6.3 and RR 0.48, 95% CI 0.27, 0.83, NNT 4.6, respectively).

However, despite these apparently promising results, analysis of the only two high quality studies did not show a reduction in the use of ET (RR 0.98, 95% CI 0.48 to 1.98; NNT 284) (see Figure 4).[2,37]

Figure 4. Forest plot of the use of exchange transfusion (one or more). High quality studies only.



Overall, immunoglobulin treatment also led to a reduction in the mean number of ETs per infant (MD -0.31, 95% CI -0.45 to -0.17). In contrast, analysis of the two high quality studies showed that IVIg did not reduce the number of ETs (MD -0.04, 95% CI -0.18 to 0.10).

Secondary outcomes

Top up transfusions during and after the first week

The results of five studies could be entered in the meta-analysis of the use of top-up transfusions in the first week[2,29,32,33,37] and of 8 studies for the use of top-up transfusions after the first week of life.[2,29,31,35-37,39,40] IVIg did not increase the need for top-up transfusions during the first week (RR 1.05, 95% CI 0.65 to 1.69) or in the period after the first week (RR 1.20, 95% CI 1.00 to 1.45). IVIg also did not increase the need for top-up transfusions in the first week and after the first week of life in the following subgroups: infants with Rh incompatibility only (RR 1.08, 95% CI 0.65 to 1.77 and RR 1.09, 95% CI 0.92 to 1.28); infants with ABO incompatibility only (RR 0.80, 95% CI 0.19 to 3.38 and RR 5.02, 95% CI 0.62 to 40.67); infants born ≥37 weeks of gestation (RR 0.91, 95% CI 0.48 to 1.74 and RR 1.18, 95% CI 0.81 to 1.71); infants born <37 weeks of gestation (RR 1.39, 95% CI 0.70 to 2.73 and RR 1.24, 95% CI 0.93 to 1.67); infants treated with IVIg ≤12 hours after birth (RR 1.18, 95% CI 0.70 to 2.00 and RR 1.10, 95% CI 0.92 to 1.31); and in those infants treated with a single

dose of IVIg (RR 1.05, 95% CI 0.65 to 1.69 and RR 1.19, 95% CI 0.99 to 1.42). Although the need for top-up transfusions during the first week of life was not increased for the subgroup of infants treated with IVIg >12 hours after birth (RR 0.71, 95% CI 0.24 to 2.12), the need for top-up transfusions after the first week of life was increased with IVIg treatment (RR 8.00, 95% CI 1.03 to 62.26). However, the CI is very large and the lower CI limit is nearly one. For infants treated with multiple IVIg doses the use of top-up transfusions after the first week of life was not increased (RR 2.09, 95% CI 0.54 to 8.13) and not estimable for the first week of life. For the subgroup of infants included in high quality studies only[2,37], the need for top-up transfusions in the first week of life and thereafter was also not altered in infants treated with IVIg (RR 1.18, 95% CI 0.70 to 2.00 and RR 1.01, 95% CI 0.80 to 1.27).
Santos et al. and Smits-Wintjens et al. were the only studies included in the analysis of the number of top-up transfusions per infant.[2,37] In the first week of life and thereafter, the number of top-up transfusions was not altered in IVIg treated infants (MD 0.05, 95% CI -0.07 to 0.17 and MD -0.00, 95% CI -0.12 to 0.12, respectively).

Maximum serum bilirubin

Results for this outcome were available for nine studies.[2,29,31-34,37,39,40] The meta-analysis of all 9 studies showed that the mean maximum serum bilirubin decreased by 17.52 µmol/L in those receiving IVIg (MD -17.52, 95% CI -25.20 to -9.84). Furthermore, subgroup analyses showed that IVIg decreased maximum bilirubin levels in infants with both Rh and ABO incompatibility, those of >37 weeks of gestation, those treated early or late and those treated with a single dose of IVIg. However, subgroup analyses of the only two high quality studies[2,37], of infants born <37 weeks[2,37] and of infants treated with multiple doses of IVIg[33,39] showed that IVIg did not reduce maximum serum bilirubin (MD 0.92, 95% CI -23.94 to 25.79; MD -18.91, 95% CI -54.49 to 16.68; and MD 22.89, 95% CI -12.00 to 57.78, respectively).

Duration of phototherapy

Results of ten studies could be included in the meta-analysis of the duration of phototherapy.[2,29,30,32-37,41] Although criteria for commencing phototherapy were given in all studies except for the study by Hematyar et al.[34], only five studies described or provided predefined criteria for ceasing phototherapy.[2,29,32,37,41] Analysis of all ten studies showed that duration of phototherapy decreased by 0.87 days with IVIg treatment (MD -0.87, 95% CI -1.24 to -0.50). All subgroup analyses showed a decrease in duration of phototherapy in IVIg treated infants varying from a mean decrease of 0.74 days in those infants treated with IVIg >12 hours after birth (MD -0.74, 95% CI -1.00 to -0.49) to 1.22 days (MD -1.22, 95% CI

-1.42 to - 1.01) in infants treated with IVIg ≤12 hours after birth. However, as for maximum bilirubin levels, analyses of the two high quality studies (MD -0.50 95% CI -1.24 to 0.24) and of infants born <37 weeks of gestation (MD -0.91, 95% CI -1.96 to 0.14) showed no reduction in duration of phototherapy.

Duration of hospitalization

Results of eight studies could be entered in the meta-analysis.[2,29,30,32,34-37] None of these studies described predefined criteria for hospital discharge and only three studies provided them through correspondence.[34,35,37] The analysis showed that IVIg treatment shortens duration of hospitalization by 1.50 days (MD -1.50, 95% CI -1.72 to -1.28). All subgroup analyses showed a shorter duration of hospitalization with IVIg treatment varying from a mean decrease of 0.87 days in the infants with ABO incompatibility (MD -0.87, 95% CI -1.40 to -0.35) to 2.28 days less in infants born <37 weeks of gestation (MD -2.28, 95% CI -3.84 to -0.72).

Incidence of adverse reactions

All studies, except for Hematyar et al.[34], reported or subsequently provided data on adverse reactions. Eleven studies reported that no adverse reactions of IVIg treatment were observed.[2,29,31,32,35-41] In one study five patients developed fever up to 38.5 °C after ET which spontaneously disappeared after 4-6 hours.[30] In the study by Garcia et al., two deaths/adverse events occurred in both groups.[33] Causes of death in the IVIg group were pulmonary arrest and septic shock in babies with severe HDN and in the control group two infants died of hydrops and cardiac arrest also in the setting of severe HDN.[33] In the study by Alpay et al. two control infants receiving ET developed hypoglycemia and hypocalcemia after ET.[29] In the Rübo 1992 study, one control infant who required ET developed sepsis and one control infant who required ET developed inspissated bile syndrome.[40] However, the authors stated that a causal relationship with ET could not be established in either infant. Also in the study by Dağoğlu et al., one control infant developed inspissated bile syndrome.[31] Miqdad et al. described that “no immediate adverse effects related to IVIg were noted, including fever, allergic reactions, volume overload or hemolysis”, however they also stated that “ten of the babies who had ET, from both groups, had to be treated for blood culture-positive or clinical sepsis”.[35] In the study by Smits-Wintjens et al. one infant from the IVIg group developed a Bacillus cereus sepsis with brain abscesses a few days after ET.[2] Sterility tests on the used IVIg batches and cultures of all donor blood products used for IUTs and ET were found to be sterile. The sepsis may have been related to the umbilical venous catheterization and ET. Detailed information on this exceptional case is described in a case report.[47]

Long-term outcomes

Only the studies by Santos et al. and Miqdad et al. had a relatively long follow up period of one year and two years, respectively.[35,37] In both studies no cases of kernicterus, deafness or cerebral palsy were observed.

Discussion

Summary of main results

Overall there is limited evidence that IVIg treatment in neonates with alloimmune HDN reduces the need for ET. Although this review update showed a significant reduction in the need for ET, most of the included studies were not of high quality. In contrast, subgroup analysis of the only two studies which were of high quality showed that IVIg treatment had no effect on the need for ET or the number of ETs per infant. IVIg treatment was also associated with a significant reduction in maximum bilirubin level and duration of phototherapy when all included studies were analyzed as well as for most of the subgroup analyses based on type of alloimmunization, gestational age at birth and timing and number of doses of IVIg. However, as for ET, analysis of the two high quality studies demonstrated no difference in maximum bilirubin level and duration of phototherapy. Duration of hospitalization was significantly reduced when analyzing all studies which reported this outcome and for almost all subgroup analyses, including the subgroup analysis of high quality studies only. Although there is some evidence that IVIg reduces hemolysis and shortens hospital stay, these results should be interpreted with caution because only three studies used predefined criteria for hospital discharge and criteria for stopping phototherapy were not reported in most studies. In addition, over the last two decades guidelines for phototherapy have recommended using it more promptly for infants at risk of hemolysis.[48] In many hospitals, the quality of phototherapy has also improved over the years. Nevertheless, the quality/intensity of phototherapy can still vary today, especially in low-resource settings and if good quality control is not applied. The incidence of late top-up transfusions is an important outcome, especially in areas where follow-up of infants is difficult or where supply of safe blood for transfusion is limited. However, as thresholds for top-up transfusions in neonates vary widely, this outcome is susceptible to bias, particularly in unblinded studies. Eight of fourteen studies were included in the analysis of the incidence of top-up transfusion after the first week of life. However, only five of the eight studies used predefined criteria for top-up transfusions. In addition, those predefined criteria varied between studies, thus conclusions that could be drawn for this outcome are limited. Data on adverse events of IVIg seem to indicate that it can be used safely, although it is unclear

whether IVIg contributed to the two deaths in the IVIg study arm in the study by Garcia et al.[33]

Overall completeness and applicability of evidence

This review included all (quasi-)RCTs on the use of IVIg in alloimmune HDN. Nineteen trials were identified, of which fourteen trials, comprising a total of 942 infants, fulfilled inclusion criteria for the review. The only two included studies that were of high quality comprising a total of 172 infants, enrolled only infants with Rh HDN and the intervention consisted of a single dose of 0.5-0.75 g/kg IVIg administered within 4-6 hours after birth.[2,37] Santos et al. included infants of ≥32 gestational weeks and Smits-Wintjens et al. included infants of ≥35 gestational weeks.[2,37] Criteria for phototherapy and ET were similar in both studies. From subgroup analysis of these two studies, it can be concluded that early administration of IVIg in a single dose of 0.5-0.75 g/kg does not reduce ETs or have other benefits in the treatment of Rh HDN. There is no clear evidence from this review that a higher dose will improve the efficacy. The only randomized controlled trial comparing the effect of two doses of IVIg in Rh HDN showed that 0.5 g/kg and 1 g/kg had a similar effect on the duration of phototherapy, duration of hospitalization and ET requirements.[44] However, this study was not powered to find a difference in the need for ET. Long-term neurodevelopmental outcome was only examined by Santos et al. and Miqdad et al. who found no cases of kernicterus, deafness or cerebral palsy in a follow up period of one year and two years, respectively.[35,37]

Subgroup analysis of infants with ABO HDN showed a reduction in ET, maximum serum bilirubin level and duration of phototherapy and hospitalization. However, none of the four studies was of high quality and results may be limited in applicability.[29,35,36,38]

American Academy of Pediatrics guidelines of 2004 recommend the administration of 0.5-1 g/kg IVIg in alloimmune HDN if TSB is rising despite intensive phototherapy or if TSB level is within 34-51 µmol/l (2-3 mg/dL) of exchange level.[27] Based on the results of this review and because IVIg administration is not completely without risks[20-22] and supplies of IVIg are limited, we do not recommend routine use of IVIg. However, since there is some evidence that it reduces hemolysis and it appears safe in infants with alloimmune HDN, it might be reasonable to consider using it in special circumstances, such as during transfer of an infant to a location that can perform an ET, or where the risk of ET is considered to be much higher than usual, such as in very or extremely low birth weight infants.

Quality of the evidence

The quality of included studies ranged from fulfilling none of the 'risk of bias' criteria to fulfilling all criteria. Six of fourteen studies used adequate methods for random sequence generation and allocation concealment. Given the nature of the intervention, it was possible to blind patients by using an infusion fluid. However, only three of fourteen studies were placebo-controlled. The lack of blinding could have influenced the decision to perform an ET or top-up transfusion. None of the studies that were not placebo-controlled described any another method to blind outcome assessors except for Miqdad 2004 and Elalfy 2011.[32,35] Attrition was rated as high risk of bias in only one study (on the basis of both extent and reasons for attrition) and two studies were rated as unclear risk of bias because the available information was too limited to make a judgement. Selective reporting was suspected in nine studies because they did not report on duration of hospitalization and/or top-up transfusions, which could cause over- or underestimation of the overall benefits of IVIg. In addition, since ETs and top-up transfusions are related in Rh HDN, both outcome measures should be described.[49] Finally, risk of other biases was suspected in four trials which used different ET criteria for the IVIg and control groups, did not perform analysis on an intention to treat basis, or had significant non-random crossover between study groups. In conclusion, only two of fourteen trials fulfilled all criteria to be rated as high quality studies.

Potential biases in the review process

We tried to minimize bias by working with two reviewers who independently assessed eligibility for inclusion of trials, extracted data and assessed risk of bias. However, we were aware that these parts of the review process were based on personal judgement because reviewing research is influenced by prior beliefs. In addition, one included trial was performed by three of the four review authors. Nevertheless, we attempted to review all studies in a similar way. In addition, we were unable to contact authors of all potentially eligible studies and therefore we could not include all available data. While the translator of the Turkish included study was a medical doctor from Turkish parents, he may have missed some details regarding the risk of bias of that study.

Agreements and disagreements with other studies or reviews

The overall findings of this review are consistent with previous systematic reviews. Gottstein et al. included 3 studies that were also included in our review (Rübo 1992; Dağoğlu 1995; Alpay 1999) and one study that was excluded from our review (Voto 1995).[50] They concluded that with IVIg treatment significantly fewer infants required ET. Duration of

hospitalization and phototherapy were also significantly reduced in their review.[50] However, based on our judgement, none of their included studies was of high quality. Two Chinese systematic reviews also found a reduction in ET requirements, duration of phototherapy and hospitalization but concluded that well-designed trials with a larger sample size were required for further evaluation of the efficacy and safety of IVIg.[51,52] Until the date we conducted our search, our review is the most recent, extensive and up-to-date review of all randomized and quasi-randomized trials on the effect of IVIg in alloimmune HDN.

Authors' conclusions

Implications for practice

Routine use of IVIg for the treatment of alloimmune HDN should be discouraged. Results of the only two high quality studies show no reduction in the use of ET. In addition, IVIg is not without risks and supplies are limited. However, since there is some evidence that IVIg reduces hemolysis and appears safe in neonates with alloimmune HDN, it may have a limited role in special circumstances, such as where ET is impossible, or is considered particularly high risk. Nevertheless, undertaking preparations for ET, including ensuring birth at or transfer to a center that can perform ET, would seem to be strongly indicated in high risk infants, and should not be abandoned in the expectation that IVIg will be efficacious.

Implications for research

Future research into the role of IVIg in the early treatment of alloimmune HDN may be warranted, particularly in infants for whom ET is considered to be high risk. Such a trial should examine the safety and efficacy of IVIg by recording both short-term outcomes such as the need for transfusion therapy and the incidence of adverse events and also long-term neurodevelopmental outcomes. Both ETs and (late) top-up transfusions should be recorded because reduction of ETs can increase the number of top-up transfusions.[49] Consideration should also be given to including additional measures to assess the severity of hemolysis such as carboxyhemoglobin or end tidal carbon monoxide. Based on evidence from the two high quality trials, the conclusion of the review authors is that IVIg is of very limited usefulness in Rh HDN. However, neither of these high quality studies enrolled infants with severe established jaundice due to ABO incompatibility. In contrast to Rh incompatibility, ABO incompatibility mainly results in hyperbilirubinemia without significant anemia. This is primarily due to the relatively few group A and B antigenic sites on neonatal red blood cells.[53] Furthermore, infants with ABO-mediated hemolysis often present for neonatal care when they already have severe jaundice. Due to these differences between Rh and ABO

incompatibility it is conceivable that IVIg has a greater role in ABO-mediated jaundice. If it is efficacious in ABO HDN, it could, for example, be used during transfer to a hospital that can provide intensive phototherapy and perform ET. However, due to the relative rarity of severe jaundice, unresponsive to phototherapy in ABO incompatibility, exploring the use of IVIg to treat established jaundice would require a multicenter randomized controlled trial. Such a trial should either use a placebo or an alternative method for blinding of treatment and outcome assessment. Future trials should be well planned and give priority to establishing guidelines for the "conventional" management of alloimmune HDN, focusing on the criteria for performing both top-up and ETs and on the role of intensive phototherapy.

Acknowledgements

We thank Gary Alcock for writing the original protocol and review, Gürbey Ocak for his help in translating the Turkish article, David Corpman for translating the two Chinese articles, Jan Schoones for his help in making and performing the literature search, and Lizelle Weber for her contributions to the literature search.

References

1. 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 Jul;120(1):27-32.
2. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D, Brand A, Lopriore E. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011 Apr;127(4):680-6.
3. Badiee Z. Exchange transfusion in neonatal hyperbilirubinaemia: experience in Isfahan, Iran. Singapore Med.J. 2007 May;48(5):421-3.
4. Boggs T.R., Westphal M.C.Jr. Mortality of exchange transfusion. Pediatrics 1960;26:745-55.
5. Guaran RL, Drew JH, Watkins AM. Jaundice: clinical practice in 88,000 liveborn infants. Aust.N.Z.J.Obstet.Gynaecol. 1992 Aug;32(3):186-92.
6. Jackson JC. Adverse events associated with exchange transfusion in healthy and ill newborns. Pediatrics 1997 May;99(5):E7.
7. Keenan WJ, Novak KK, Sutherland JM, Bryla DA, Fetterly KL. Morbidity and mortality associated with exchange transfusion. Pediatrics 1985 Feb;75(2 Pt 2):417-21.
8. Panagopoulos G, Valaes T, Doxiadis SA. Morbidity and mortality related to exchange transfusions. J.Pediatr. 1969 Feb;74(2):247-54.
9. Patra K, Storfer-Isser A, Siner B, Moore J, Hack M. Adverse events associated with neonatal exchange transfusion in the 1990s. J.Pediatr. 2004 May;144(5):626-31.
10. Ip S, Chung M, Kulig J, O'Brien R, Sege R, Glicken S, Maisels MJ, Lau J. An evidence-based review of important issues concerning neonatal hyperbilirubinemia. Pediatrics 2004 Jul;114(1):e130-e153.
11. Hosseinpour SS, Gharehbaghi MM. Exchange transfusion in severe hyperbilirubinemia: an experience in northwest Iran. Turk.J.Pediatr. 2010 Jul;52(4):367-71.
12. Davutoglu M, Garipardic M, Guler E, Karabiber H, Erhan D. The etiology of severe neonatal hyperbilirubinemia and complications of exchange transfusion. Turk.J.Pediatr. 2010 Mar;52(2):163-6.
13. Hara T, Mizuno Y, Kawano M, Ueki Y, Ueda K. Treatment of immune hemolytic anaemia with gammaglobulin. J.Pediatr. 1987;(110):817-8.
14. Ergaz Z, Arad I. Intravenous immunoglobulin therapy in neonatal immune hemolytic jaundice. J.Perinat.Med. 1993;21(3):183-7.
15. Kubo S, Ariga T, Tsuneta H, Ishii T. Can high-dose immunoglobulin therapy be indicated in neonatal rhesus haemolysis? A successful case of haemolytic disease due to rhesus (c + E) incompatibility. Eur.J.Pediatr. 1991 May;150(7):507-8.
16. Sato K, Hara T, Kondo T, Iwao H, Honda S, Ueda K. High-dose intravenous gammaglobulin therapy for neonatal immune haemolytic jaundice due to blood group incompatibility. Acta Paediatr. Scand. 1991 Feb;80(2):163-6.
17. Hammerman C, Kaplan M, Vreman HJ, Stevenson DK. Intravenous immune globulin in neonatal ABO isoimmunization: factors associated with clinical efficacy. Biol.Neonate 1996;70(2):69-74.
18. Brocklehurst P, Farrell B, King A, Juszczak E, Darlow B, Haque K, Salt A, Stenson B, Tarnow-Mordi W. Treatment of neonatal sepsis with intravenous immune globulin. N.Engl.J.Med. 2011 Sep 29;365(13):1201-11.

19. Fischer GW. Therapeutic uses of intravenous gammaglobulin for pediatric infections. Pediatr.Clin. North Am. 1988 Jun;35(3):517-33.
20. Copelan EA, Strohm PL, Kennedy MS, Tutschka PJ. Hemolysis following intravenous immune globulin therapy. Transfusion 1986 Sep;26(5):410-2.
21. Magny JF, Bremard-Oury C, Brault D, Menguy C, Voyer M, Landais P, Dehan M, Gabilan JC. Intravenous immunoglobulin therapy for prevention of infection in high-risk premature infants: report of a multicenter, double-blind study. Pediatrics 1991 Sep;88(3):437-43.
22. 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 Jan;125(1):139-44.
23. Kumar A, Teuber SS, Gershwin ME. Intravenous immunoglobulin: striving for appropriate use. Int. Arch.Allergy Immunol. 2006;140(3):185-98.
24. Urbaniak SJ. ADCC (K-cell) lysis of human erythrocytes sensitized with rhesus alloantibodies. II. Investigation into the mechanism of lysis. Br.J.Haematol. 1979 Jun;42(2):315-25.
25. Ergaz Z, Gross D, Bar-Oz B, Peleg O, Arad I. Carboxyhemoglobin levels in neonatal immune hemolytic jaundice treated with intravenous gammaglobulin. Vox Sang. 1995;69(2):95-9.
26. Hammerman C, Vreman HJ, Kaplan M, Stevenson DK. Intravenous immune globulin in neonatal immune hemolytic disease: does it reduce hemolysis? Acta Paediatr. 1996 Nov;85(11):1351-3.
27. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004 Jul;114(1):297-316.
28. Higgens J.P.T., Green S, (editors). Cochrane handbook for systematic reviews of interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration; 2011.Available from www.cochrane-handbook.org
29. Alpay F, Sarici SU, Okutan V, Erdem G, Ozcan O, Gokcay E. High-dose intravenous immunoglobulin therapy in neonatal immune haemolytic jaundice. Acta Paediatr. 1999 Feb;88(2):216-9.
30. Atici A, Satar M, Göðebakan M. Intravenous immunoglobulin therapy for neonatal hyperbilirubinemia due to ABO or Rh incompatibility. [ABO veya Rh Uyuþmazliðinin neden olduðu neonatal hiperbilirübinemide intravenöz immüglobülin tedavisi.]. Çocuk Saðliði ve Hastaliklari Dergisi 1996;39:623-30.
31. Dagoglu T, Ovali F, Samanci N, Bengisu E. High-dose intravenous immunoglobulin therapy for rhesus haemolytic disease. J.Int.Med.Res. 1995 Jul;23(4):264-71.
32. Elalfy MS, Elbarbary NS, Abaza HW. Early intravenous immunoglobin (two-dose regimen) in the management of severe Rh hemolytic disease of newborn--a prospective randomized controlled trial. Eur.J.Pediatr. 2011 Apr;170(4):461-7.
33. Garcia MG, Cordero G, Mucino P, Salinas V, Fernandez LA, Christensen RD. Intravenous Immunoglobulin (IVIG) Administration as a Treatment for Rh Hemolytic Jaundice in Mexico City. Pediatr.Res. 55, 65. 2004.
34. Hematyar M, Zareian M. The effects of intravenous immunoglobulin(IVIG) in hemolytic jaundice of the newborn due to ABO and Rh isoimmunization. Acta Paediatrica 100[Suppl. 463], 71. 2011.
35. Miqdad AM, Abdelbasit OB, Shaheed MM, Seidahmed MZ, Abomelha AM, Arcala OP. Intravenous immunoglobulin G (IVIG) therapy for significant hyperbilirubinemia in ABO hemolytic disease of the newborn. J.Matern.Fetal Neonatal Med. 2004 Sep;16(3):163-6.
36. Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. Saudi.Med.J. 2006 Dec;27(12):1827-30.

37. Pessoa dos Santos M.C., Amaral Moura Sá C., Gomes S.C.Jr., Gamacho L.A., Lopes Moreira M.E. High-dose intravenous immunoglobulintherapy for hyperbilirubinemia due rhhemolytic disease: a randomized clinical trial. Vox Sang 2011; 101 (Suppl. 1), 291-292.
38. Pishva N, Madani A, Homayoon K. Profylactic intravenous immunoglobulin in neonatal immune hemolytic jaundice. Iranian Journal of Medical Sciences 2000;25:129-33.
39. Rübo J, Wahn V. Influence of high dosage immuno-globulin therapy on hyperbilirubinemia in rhesus-hemolytic disease. A cooperative study. [Kooperative Studie zum Einfluß einer hochdosierten Immunglobulintherapie auf die Hyperbilirubinämie bei Rhesusinkompatibilität.]. Monatsschrift fur Kinderheilkunde 1996;144:516-9.
40. Rübo J, Albrecht K, Lasch P, Laufkotter E, Leititis J, Marsan D, Niemeyer B, Roesler J, Roll C, Roth B, et al. High-dose intravenous immune globulin therapy for hyperbilirubinemia caused by Rh hemolytic disease. J.Pediatr. 1992 Jul;121(1):93-7.
41. Tanyer G, Siklar Z, Dallar Y, Yildirmak Y, Tiras U. Multiple dose IVIG treatment in neonatal immune hemolytic jaundice. J.Trop.Pediatr. 2001 Feb;47(1):50-3.
42. Huang WM, Chen HW, Li N, Yang M, Jiao PY. [Clinical study of early interventions for ABO hemolytic disease of the newborn]. Nan.Fang Yi.Ke.Da.Xue.Xue.Bao. 2006 Sep;26(9):1350-1, 1355.
43. Wang M.Q., Guo Y.X., Yu S.Q. Curative observation of ABO HDN treated by immunogbulin. Shanxi yi yao za zhi 2002;31(3):239-40.
44. Girish G, Chawla D, Agarwal R, Paul VK, Deorari AK. Efficacy of two dose regimes of intravenous immunoglobulin in Rh hemolytic disease of newborn--a randomized controlled trial. Indian Pediatr. 2008 Aug;45(8):653-9.
45. Spinelli S.L., Otheguy L.E., Larguia M.A. Postnatal use of high-dose intravenous immunoglobulin therapy in rhesus hemolytic disease treatment. J.Perinat.Med. 2001; 29 Suppl 1, 683.
46. Voto LS, Sexer H, Ferreiro G, Tavosnanska J, Orti J, Mathet ER, Margulies M, Margulies M. Neonatal administration of high-dose intravenous immunoglobulin in rhesus hemolytic disease. J.Perinat.Med. 1995;23(6):443-51.
47. Smits-Wintjens VE, Steggerda SJ, Oepkes D, Van Kamp IL, Kramer CM, Walther FJ, Lopriore E. Bacillus cereus cerebral abscesses in a term neonate with rhesus hemolytic disease treated with exchange transfusion. J.Pediatr.Inf.Dis. 2010;5(3):277-80.
48. Gartner L.M. Disorders of bilirubin metabolism. In: Nathan D.G., Oski F.A., editors. Hematology of infancy and childhood. 3rd ed. Philadelphia: Saunders; 1987. p. 92.
49. Rath ME, Smits-Wintjens VE, 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(1):65-70.
50. Gottstein R, Cooke RW. Systematic review of intravenous immunoglobulin in haemolytic disease of the newborn. Arch.Dis.Child Fetal Neonatal Ed 2003 Jan;88(1):F6-10.
51. Li M.J., Chen C.H., Wu Q, Shi W, Yang Q, Li M.N. Intravenous immunoglobulin G for hemolytic disease of the newborn: A systematic review. Chinese Journal of Evidence-Based Medicine 2012;10:1199-204.
52. Li ZH, Wang J, Chen C. [Meta analysis of the effect of immunoglobulin infusion on neonatal isoimmune hemolytic disease caused by blood group incompatibility]. Zhonghua Er.Ke.Za Zhi 2010 Sep;48(9):656-60.
53. Murray NA, Roberts IA. Haemolytic disease of the newborn. Arch.Dis.Child Fetal Neonatal Ed 2007 Mar;92(2):F83-F88.

54. Brouwers HA, Overbeeke MA, Huiskes E, Bos MJ, Ouwehand WH, Engelfriet CP. Complement is not activated in ABO-haemolytic disease of the newborn. Br.J.Haematol. 1988 Mar;68(3):363-6.
55. Maisels MJ. Jaundice. In: Avery GB. Fletcher MA, MacDonald MG, editor(s). Neonatology: pathophysiology and management of the newborn. 4th edition. Philadelphia: JB Lippincott, 1994.
56. Oski FA, Naiman JL. Erythroblastosis fetalis. In: Oski FA, Naiman JL, editor(s). Hematological problems in the newborn. Philadelphia: Saunders WB, 1982: 283-346.
57. Fanaroff A, Martin RJ, Miller MJ. Identification and management of high-risk problems in the neonate. In: Creasy PK, Renisk R, editor(s). Maternal-Fetal Medicine (Principles and Practice). 3rd edition. Philadelphia: Saunders WB, 1994:1158.
58. Polacék K. The early indications for exchange transfusion in hemolytic disease of the newborn [Die frühzeitige Indikationstellung zur Austauschtransfusion bei hämolytischen Neugeborenenerkrankungen]. Monatsschrift Kinderheilkunde 1963; 111:6-10.
59. Polacék K. The universal diagram for the treatment of hyperbilirubinemia of the newborn [Das universale Diagramm zur Behandlung der Hyperbilirubinämie der Neugeborenen]. Pädiatrische Praxis 1984; 29:1-3.
60. Santos MC, Sa C, Gomes Jr 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 2012 Aug 6. doi: 10.1111/j.1537-2995.2012.03827.x.:[Epub ahead of print].
61. Bryla DA. Randomized, controlled trial of phototherapy for neonatal hyperbilirubinemia. Development, design, and sample composition. Pediatrics 1985; 75(2 pt 2):387-92.

Appendix 1: Details of included studies

Alpay 1999[29]

Methods	RCT
Participants	116 newborn term infants. ABO and/or Rh incompatibility. TSB >204 µmol/L (12 mg/dL), positive direct Coombs test and reticulocyte count ≥10%.
Interventions	Treatment group: Single dose of IVIg 1 g/kg (ISIVEN) plus phototherapy, started at 51.53 ± 3.5 h (mean ± SD) after birth. (n = 58) Contol group: Phototherapy only, started 54.33 ± 4.0 h (mean ± SD) after birth. (n = 58)
Outcomes	ETs, maximum TSB*, duration of phototherapy, duration of hospitalization, top-up transfusions*, and adverse events. Criteria for ET: TSB >290 µmol/L (17 mg/dL) and increased by >17 µmol/L/hour (1 mg/dL/hour). Criteria for phototherapy: started and continued as long as TSB levels were above the levels for starting phototherapy.[55] Details of phototherapy: 5 special blue lights (Philips F20 T12/BB) placed 30 cm above the patient; body position was changed periodically; no phototherapy blanket. Criteria for top-up transfusions: after 15-21 days red blood cell transfusions were given because hemoglobin levels were ≤87 g/L . * = (part of the) outcome available through correspondence
Notes	Unpublished data and information supplied.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not stated. Method of randomization unclear despite correspondence with author.
Allocation concealment (selection bias)	Unclear risk	Only stated: "The attending neonatologists who made the decision regarding the choice of treatment were different from those conducting the study."
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.

Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	Low risk	Although adverse events of IVIg were not reported explicitly, assumed that there were no adverse events of IVIg because authors described that 2 patients had hypoglycemia and hypocalcemia after ET.
Other bias	Unclear risk	Average bilirubin levels at study entry were already above bilirubin thresholds to invoke outcome event ET. Possible sources of bias include: 1) dilution effect of IVIg could have affected bilirubin after infusion, 2) rate of rise of bilirubin might have been measured over different intervals, 3) decision to prepare for ET might easily have been influenced by treatment group allocation, because of the urgency.

Atici 1996[30]

Methods	RCT
Participants	116 newborn term infants. ABO and/or Rh incompatibility. TSB >204 µmol/L (12 mg/dL), positive direct Coombs test and reticulocyte count ≥10%.
Interventions	Treatment group: Single dose of IVIg 1 g/kg (ISIVEN) plus phototherapy, started at 51.53 ± 3.5 h (mean ± SD) after birth. (n = 58) Contol group: Phototherapy only, started 54.33 ± 4.0 h (mean ± SD) after birth. (n = 58)
Outcomes	ETs, maximum TSB*, duration of phototherapy, duration of hospitalization and adverse events. Criteria for ET: TSB level 86 µmol/L (5 mg/dL) above the limit in Oski 1982.[56] Criteria for phototherapy: TSB level beneath the level for ET. Details phototherapy: blue light 420-470 nm. * = not presented in a form usable for meta-analysis
Notes	Not stated whether any ethics approval or if parental consent was given.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Only stated "block randomization".
Allocation concealment (selection bias)	Unclear risk	Not stated.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Low risk	3 patients appear to be missing in Table 3 of outcome data (n = 36 for control group), but this is probably a typing mistake since percentages correspond with inclusion of all patients (n = 39) in control group.
Selective reporting (reporting bias)	High risk	Top-up transfusions not reported.
Other bias	Unclear risk	Absence of strict criteria for enrolment. In addition, a positive direct antiglobulin test was not a criterion for enrolment and only present in 63% and 59% of infants in IVIg and control group, respectively. Unclear if block randomization included stratification for Rh versus ABO HDN.

Dağoğlu 1995[31]

Methods	RCT
Participants	45 term and preterm infants with Rh incompatibility randomized. Four infants withdrawn post-randomization because parental consent was not provided. Rh positive infant, Rh negative mother and positive direct Coombs test.
Interventions	Treatment group: Single dose of IVIg 0.5 g/kg (Sandoglobulin) as soon as possible after birth (usually within 2 hours) plus phototherapy. (n = 22) Control group: Only phototherapy. (n = 19)

Outcomes	ETs, maximum TSB, duration of phototherapy*, top-up transfusions and adverse events. Criteria for ET: TSB increase by >17 µmol/L/hour (1 mg/dL/hour) or TSB >342 µmol/L (20 mg/dL) in term infants or if TSB >308 µmol/L (18 mg/dL) in infants weighing >2000g. Criteria for phototherapy: started when bilirubin levels exceeded the relevant curves of Oski and Naiman (Oski 1982).[56] Details phototherapy: blue lights 420-460 nm. Criteria for top-up transfusion: not stated. * = not presented in a form usable for meta-analysis
Notes	45 infants eligible. Post-randomization consent used. All infants received at least one IUT.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Stated "random numbers".
Allocation concealment (selection bias)	Low risk	Stated "sealed envelopes".
Blinding of participants and personnel (performance bias)	High risk	Stated "not blinded because an appropriate placebo for IVIg could not be found".
Blinding of outcome assessment (detection bias)	High risk	Not stated. Contact with authors unsuccessful. Assumed that blinding was not performed.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	High risk	Duration of hospitalization not reported.
Other bias	Unclear risk	Consent after randomization and 2 infants from each group withdrawn post-randomization because consent not provided. Reasons for parental refusal are not stated. Some differences in IVIg and control groups despite randomization: higher M:F ratio in IVIg group (72%) than control group (47%) although most other characteristics don't differ. High rate of ET in control group (79%) for patients who all had IUT. ET criteria inconsistently described in Methods and Discussion.

Elalfy 2011[32]

Methods	RCT
Participants	90 term neonates (>38 weeks of gestation) born to Rh negative mothers who had not received anti-D after previous deliveries with: 1) isoimmune HDN "proven by": Rh incompatibility between blood group of the mother and baby, a positive direct antiglobulin test and a high reticulocyte count and 2) significant hyperbilirubinemia requiring phototherapy in the first 12 hours of life and/or rising by 8.6 µmol/L/hour (0.5 mg/dL/hour) while TSB is still below the ET criteria on admission according to the AAP management guidelines for hyperbilirubinemia.[27]
Interventions	Treatment group 1: Single dose of IVIg 0.5 g/kg administered at 12 hours after birth plus phototherapy. (n = 25) (Number randomized n = 23, however 3 moved to control group and 5 gained from high dose IVIg group.) Treatment group 2: Single dose of IVIg 1 g/kg administered at 12 hours after birth plus phototherapy. (n = 15) (Number randomized n = 22, however 2 moved to control group and 5 to low dose IVIg group.) Control group: phototherapy only. (n = 50) (Number randomized n = 45, however 5 gained from IVIg groups)
Outcomes	ETs, duration of phototherapy, top-up transfusions*, duration of hospitalization and adverse events. Criteria for ET: "When bilirubin increased by 17 µmol/L/hour (1 mg/dL/hour), the neonate will require ET according to the guidelines of the AAP".[27] Criteria for phototherapy: "Initiation and discontinuation of phototherapy was according to the serum bilirubin levels as provided by the AAP guidelines".[27] Phototherapy details: 5 special blue lights, of which one fiberoptic blanket and 4 overhead lights. Criteria top-up transfusion: not stated. * = information on this outcome through correspondence.
Notes	Unpublished data and information supplied. Follow up until one week after discharge.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	From correspondence: randomization by using sealed envelopes which were kept in a big box and shuffled. A neonatologist picked one envelop out of the box to randomize a participant.
Allocation concealment (selection bias)	Low risk	See above.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	Low risk	No placebo or other method of blinding described. An e-mail reply to correspondence stated that the study was blinded and there was no detection bias, but does not state what methods were used. The authors explained that they meant by "the study was blinded" that the person who performed the randomization was different from the one who conducted the study and the one who analyzed the data.
Incomplete outcome data (attrition bias)	High risk	Substantial amount of missing data for bilirubin levels after 48 hours (figure 2 in article).
Selective reporting (reporting bias)	High risk	Top-up transfusions were not reported in the paper, but data on the number of top-up transfusions in the first week and thereafter were provided through correspondence. However, duration of follow-up was only until one week after discharge from the hospital, therefore top-up transfusions after the first week are still missing.
Other bias	High risk	Significant non-random crossover between study groups after randomization, quote: "...five parents in the intervention group did not consent using IVIg, so they were treated eventually by the conventional method. Of the 40 infants finally in the intervention group, five babies assigned to the higher IVIg dose... their parents chose the lower dose...". Authors explained through correspondence that when parents signed the informed consent form, they had the right to change the treatment without knowing in which arm their child was randomized. It happened to be that all parents who changed the treatment were initially randomized to the low dose arm.

Garcia 2004[33]

Methods	Double blind, placebo controlled RCT (pilot study)
Participants	18 neonates with jaundice due to Rh incompatibility and who required phototherapy.
Interventions	Treatment group: IVIg 0.75 g/kg/day (15 ml/kg/day) for 3 days started 2 h (mean) after birth plus phototherapy. (n = 11) Control group: An equal amount (15 ml/kg/day) of saline started 2 h (mean) after birth with the same frequency plus phototherapy. (n = 7)
Outcomes	ETs, maximum TSB*, duration of phototherapy, top-up transfusions*, adverse events* and mortality* Criteria for ET: based on a local protocol ("Instituto Nacional de Perinatologia. Incompatibilidad al Antigeno Rh. Normas y procedimientos de Neonatologica 1998.") which was based on the guidelines from the AAP. Criteria for phototherapy: started when infants were enrolled and based on local protocol (see above). Details phototherapy: Blue lights were used, one light for each patient. Criteria for top-up transfusion: not stated. * = information on this outcome through correspondence.
Notes	Unpublished data and information supplied. Ethics approval and parental consent was given. Follow up until discharge.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	From correspondence: By a random number table.
Allocation concealment (selection bias)	Low risk	From correspondence: The random number table was managed by a secretary from the unit and each time a Rh negative woman was in the operating room for a caesarean section she informed the pharmacy which delivered the study medication labeled only with "Group 1" or "Group 2".
Blinding of participants and personnel (performance bias)	Low risk	A placebo was used and study medication only labeled with "Group 1" or "Group 2".

Blinding of outcome assessment (detection bias)	Low risk	A placebo was used and study medication only labeled with “Group 1” or “Group 2”.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes in abstract or after correspondence.
Selective reporting (reporting bias)	High risk	Duration of hospitalization not measured.
Other bias	Unclear risk	Three infants were withdrawn post-randomization because their blood type was Rh negative. Very small numbers of patients in each group. Because only one light was used per patient, phototherapy seems unlikely to have been intensive.

Hematyar 2011[34]

Methods	RCT
Participants	80 newborns of >32 weeks of gestation and birth weight >2500 gram who developed jaundice on the first postnatal day due to ABO or Rh HDN with evidence of hemolysis (positive Coombs’ test, reticulocytes >10%, Hb or Ht decline) without other causes of jaundice. Exclusion criteria were: a serious condition such as hemodynamic instability and asphyxia, indication of ET at birth, fetal hydrops, congestive cardiac dysfunction and occurrence of serious IVIg complications.
Interventions	Treatment group: 0.5 g/kg IVIg up to a maximum of 3 doses, if it was required, plus phototherapy. The first dose was administered after 12 hours after birth in all infants. (n = 39) Control group: phototherapy only. (n = 41)
Outcomes	ETs*, maximum TSB*, duration of phototherapy*, and duration of hospitalization*. Criteria for phototherapy: not provided (additional information has been requested). Criteria for ET: according to the AAP guidelines.[27] Criteria for hospital discharge: “When TSB levels did not significantly increased 24 hours after terminating phototherapy.” * = information on this outcome through correspondence.
Notes	Follow up until discharge. The study was approved by an ethical committee and all parents gave informed consent.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	From correspondence: "By a random number."
Allocation concealment (selection bias)	Unclear risk	Not enough information provided to make a judgement. Additional information has been requested.
Blinding of participants and personnel (performance bias)	High risk	Not blinded.
Blinding of outcome assessment (detection bias)	High risk	Not blinded.
Incomplete outcome data (attrition bias)	Unclear risk	Not enough information to make a judgement. Additional information has been requested.
Selective reporting (reporting bias)	High risk	Top-up transfusions, adverse events and number of ETs per infant not reported.
Other bias	High risk	Occurence of serious IVIg complications was an exclusion criterion and analyses were not performed on an intention to treat basis.

Miqdad 2004[35]

Methods	RCT
Participants	112 neonates with "significant hyperbilirubinemia due to ABO HDN confirmed by a positive Coombs' test".
Interventions	Treatment group: single dose of IVIg 0.5 g/kg plus phototherapy. (Nine patients received IVIg <12 h and 47 patients >12 h after birth). (n = 56) Control group: phototherapy only. (n = 56)

Outcomes	ETs, duration of phototherapy, duration of hospitalization*, top-up transfusions, and adverse events. Criteria for phototherapy: TSB rising by 8.5 µmol/L/hour (0.5 mg/dL/hour) or TSB >170 µmol/L (10 mg/dL) at <12 hours after birth, TSB >204 µmol/L (12 mg/dL) at <18 hours after birth or TSB >238 µmol/L (14 mg/dL) at 24 hours after birth. Phototherapy was discontinued when TSB was <205 µmol/L (12 mg/dL). Details of phototherapy: Blue fluorescent lights were used (Ameda, Switzerland and Airshields, USA). Each unit had 4 lights at wavelength 460 nm. During the study they used one unit to denote single phototherapy, two units to denote double phototherapy and three units for triple phototherapy. These were placed 35-40 cm above the infant. They did not use phototherapy blankets. Criteria for ET: if at any time TSB ≥340 µmol/L (20 mg/dL), in any of the two groups, or if it was rising by ≥8.5 µmol/L/hour (0.5 mg/dL/hour) in the neonates in the control group. Criteria for top-up transfusions: Stated that no transfusions were performed because hemoglobin levels remained >100 g/L. Criteria for hospital discharge: TSB levels not increasing 24 hours after terminating phototherapy, no feeding problems and nursing staff and parents satisfied with discharge. * = measure of variance through correspondence
Notes	Study was approved by their hospital research committee. However, it was not clear from correspondence whether parental consent was given for this study. Additional correspondence: At the time they conducted the trial in Saudi Arabia there was resistance of parents and patients to consent to research in general because of misconception that patients would not receive appropriate treatment if they were included in research projects. However, “now that there is a body governing medical practice things are changing and research now requires approval by the institute and consent of the patient or guardian.”

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	From correspondence: Randomization was done by simple sampling randomization. The first 10 participants who were numbered 1,4,7 and 10 were assigned to the IVIg group and those numbered 2,3,5,6,8 and 9 were assigned to the control group. The second group of 10 participants who were numbered 1,4,7 and 10 were assigned to the control group and those numbered 2,3,5,6,8 and 9 to the IVIg group. This sequence continued alternating between the groups until they reached 110 participants and the final 2 participants were assigned to the IVIg group so that each group consisted of 56 patients.
Allocation concealment (selection bias)	Low risk	From correspondence: Random number table was kept by the head nurse and none of the treating physicians were involved in the randomization process.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	Low risk	All data were kept and entered to their database by personnel who were not involved in the management of the cases and that data was given to the outcome assessors.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	Low risk	All relevant outcomes reported.
Other bias	High risk	Control group has an additional criterion to perform ET which could have resulted in more ETs in the control group. Very high rate of ET for ABO HDN in both study groups, especially control group. Very high rate of clinical or culture positive sepsis in neonates who had ET. Not clear whether neonates in each group were enrolled at similar post-natal age. Unsubstantiated claim in conclusions that IVIg works even when given up to 72 hours of age. No data presented to support whether late vs early administration influenced efficacy.

Nasseri 2006[36]

Methods	RCT (although in Methods stated that it was a prospective case control study).
Participants	34 neonates with: 1) gestational age of ≥37 weeks; 2) a positive direct Coombs' test due to Rh or ABO incompatibility; 3) significant hyperbilirubinemia as defined by bilirubin rising by ≥0.5 mg/dL/hour (8.5 µmol/L/hour); 4) bilirubin below ET criterion on admission; and 5) "no other risk factors such as sepsis, G6PD deficiency".
Interventions	Treatment group: 3 doses of 0.5 g/kg IVIg 12 hours apart within 2-4 hours of admission (average age at admission about 20 hours) plus phototherapy. (n = 17) Control group: Only phototherapy. (n = 17)
Outcomes	ETs, duration of phototherapy, duration of hospitalization, top-up transfusions, and incidence of adverse events. Criteria for phototherapy: "Phototherapy was started once the baby was admitted to the NICU". Details phototherapy: "double surface blue light phototherapy". Criteria for ET: Bilirubin ≥342 µmol/L (20 mg/dL) or rising by 17 µmol/L/hour (1 mg/dL/hour). Criteria for top-up transfusions: hemoglobin level <70 g/L.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not stated.
Allocation concealment (selection bias)	Unclear risk	Not stated.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	Low risk	All relevant outcomes reported.
Other bias	Unclear risk	Treatment group with multiple doses received a relatively large dose of IVIg (1.5 g/kg in the first 26-28 hours of life). This might cause a dilutional effect on bilirubin levels and therefore influence the decision for ET.

Pishva 2000[38]

Methods	RCT
Participants	40 neonates with Rh or ABO incompatibility. Inclusion criteria: a) Rh positive neonates, b) History of Rh positive sibling(s), c) maternal blood group O, d) Positive direct Coombs' test, both A and B in Rh negative mothers.
Interventions	Treatment group: single dose of IVIg 0.5 g/kg (Sandoglobulin) in the first 24 hours of life plus phototherapy. (n = 20) Control group: phototherapy only. (n = 20)
Outcomes	ETs, maximum TSB*, duration of phototherapy*, duration of hospitalization*, and adverse events. Criteria for phototherapy and ET: based on postnatal age (hours), birth weight and the level of bilirubin according to Faranoff et al.[57] * = not presented in a form usable for meta-analysis
Notes	Not stated whether any ethics approval was given.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not stated.
Allocation concealment (selection bias)	Unclear risk	Not stated.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Unclear risk	Not stated whether any attrition from study.
Selective reporting (reporting bias)	High risk	Top-up transfusions not reported.
Other bias	Unclear risk	Seems likely to have been only a mildly affected group of infants - using conservative criteria for phototherapy and ET, rates of these treatments were very low even in the control group.

Rübo 1992[40]

Methods	RCT
Participants	34 newborn infants. Rh incompatibility. Rh positive infant, Rh negative mother and positive direct Coombs' test.
Interventions	Treatment group: Single dose of IVIg 0.5 g/kg (Polyglobin N) as soon as Rh status confirmed, plus phototherapy. (n = 17) Control group: Phototherapy only. (n = 17)
Outcomes	ETs, maximum TSB, duration of phototherapy*, top-up transfusions and adverse events. Criteria for ET: TSB 34 µmol/L (2 mg/dL) > modified curve of Polácek.[58-59] Criteria for phototherapy: TSB 68 µmol/L (4 mg/dL) < modified curve of Polácek.[58-59] Details phototherapy: performed with "quartz lamps or blue light". Criteria for top-up transfusion: not stated. * = not presented in a form usable for meta-analysis
Notes	Two infants were excluded post randomization because of unspecified "protocol violations". Authors contacted. No further information available.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not stated.
Allocation concealment (selection bias)	Unclear risk	Not stated.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Low risk	Not stated, but probably data complete for all 32 infants who could be analyzed.
Selective reporting (reporting bias)	High risk	Duration of hospitalization not reported.

Other bias	Unclear risk	Two post-randomization withdrawals (one from each group) because of protocol violations. Insufficient data to determine that the 2 groups were similar at enrolment (e.g. with respect to post-natal age, gender, gestation, serum bilirubin). Described that two infants in IVIg group who needed an ET were treated suboptimally. Different treatment in this unblinded study?

Rübo 1996[39]

Methods	Multicenter RCT
Participants	74 neonates with HDN due to Rh, Kell or Duffy incompatibility proven by a positive direct Coombs' test.
Interventions	Treatment group 1: single dose of IVIg 0.5 g/kg (Polyglobin N) immediately started after Coombs' test was known to be positive plus phototherapy. (n = 28) Treatment group 2: as group 1 but with a second dose of IVIg administered after 48 hours after the first dose. (n = 25) Control group: phototherapy only. (n = 21)
Outcomes	ETs, maximum TSB, duration of phototherapy, top-up transfusions and adverse events. Criteria for phototherapy: phototherapy was given when bilirubin levels were 68 µmol/L (4 mg/dL) or less below predefined levels of ET. Criteria for ET: if bilirubin levels were ≥34 µmol/L (2 mg/dL) above the modified curves from Polácek (reference 7 and 8 of article). Criteria for top-up transfusion: not stated.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not stated.
Allocation concealment (selection bias)	Unclear risk	Not stated.

Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	High risk	Duration of hospitalization not reported and for duration of phototherapy only mentioned that it was not significantly different between the 3 groups without providing the data.
Other bias	High risk	Protocol violations led to 2 post-randomization withdrawals (unclear from what group(s)) and 4 control group infants being treated with IVIg and subsequently included in analysis (not intention to treat analysis). Stated that the treatment was conducted in accordance to guidelines of the individual hospitals. Risk of bias possible if participating hospitals did not provide the same number of patients to each study group. At admission, the control group had a significantly higher bilirubin level than the two IVIg groups.

Santos 2011[37,60]

Methods	Double blind, placebo controlled RCT
Participants	92 neonates: 1) born to Rh D negative woman with anti-Rh D antibodies; 2) of gestational age ≥32 weeks; 3) with Rh D positive blood type; and 4) with positive direct Coombs' test.
Interventions	Treatment group: single dose of IVIg 0.5 g/kg (Immunglobulin®) in the first 6 hours of life plus phototherapy. (n = 46) Control group: Saline in corresponding volume as IVIg (10 ml/kg) plus phototherapy. (n = 46)

Outcomes	ETs, maximum TSB*, top-up transfusions*, duration of phototherapy, duration of hospitalization, sensorineural hearing loss*, kernicterus*, mortality*, and adverse events. Criteria for phototherapy: Started in the first hours of life and discontinued when bilirubin level fell below 10 mg/dL after 2 days of life. Phototherapy details: high intensity phototherapy (irradiance >30 IW/cm²/nm) with special blue fluorescent light (Bili-berço, model 006/FB, FANEM, São Paulo, Brazil), halogen lamp (Bilispot, model 006/BP, FANEM); irradiance level checked prior to initiation of phototherapy using a FANEM radiometer, model 2620. Criteria for ET: bilirubin level ≥340 µmol/L (20 mg/dL) or rising by ≥8.5 µmol/L/hour (0.5 mg/dL/hour). Criteria for top-up transfusions: hemotocrit <25% with positive direct or indirect Coombs' test; hematocrit <21% with negative Coombs' test and reticulocytes <1%; hematocrit <30% with clinical signs of severe anemia (lethargy, dyspnea, feeding problems, need for oxygen, failure to thrive). Criteria for hospital discharge: gestational age >34 weeks, absence of clinical signs of anemia, bilirubin level <10 mg/dL and decreasing, ability to suck without tiring. * = information available through correspondence and from paper published after our search on 22 March 2012.[60]
Notes	Unpublished data and information supplied.

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer generated randomization in blocks of 4 with a 1:1 allocation was performed by a statistician.
Allocation concealment (selection bias)	Low risk	Statistician was responsible for concealment and opaque envelopes were used.
Blinding of participants and personnel (performance bias)	Low risk	The medication was prepared by the pharmacist and applied such that the parents, nurses and pediatricians were blinded to its identity (IVIg versus placebo).
Blinding of outcome assessment (detection bias)	Low risk	Intervention blinded as described above and investigators and treating clinicians were different groups.

Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	Low risk	Data on top-up transfusions were provided after correspondence but reason for not including data (journal advised to remove that information) in the report was reasonable and therefore classified as low risk of bias.

Smits-Wintjens 2011[2]

Methods	Double blind, placebo controlled RCT
Participants	80 neonates of ≥35 weeks of gestation. Rh HDN with positive direct Coombs' test caused by anti-D or -c antibodies of Rh D negative or Rh c negative mother. Maternal antibody-dependent cellular cytotoxicity test >50% (comparable with titer 1:64)
Interventions	Treatment group: Single dose of IVIg 0.75 g/kg (Nanogam, Sanquin, The Netherlands) within first 4 hours after birth, plus intensive phototherapy. (n = 41) (Also stratified for treatment with intrauterine transfusion.) Control group: Placebo 5% glucose infusion plus phototherapy. (n = 39)
Outcomes	ETs, maximum TSB, duration of phototherapy, duration of hospitalization, top-up transfusions* and adverse events. Criteria for ET: according to AAP 2004 guidelines[27] TSB > threshold or rate of rise of TSB >8.5 µmol/L/hour (0.5 mg/dL/hour) despite intensive phototherapy, or clinical symptoms of acute bilirubin encephalopathy. Criteria for phototherapy: started when infants were admitted and continued according to AAP 2004 guidelines.[27] Phototherapy details: intensive phototherapy using white light with an intensity of 10-20 µW/cm/nm given by Air Shield and Ohmeda lamps, in combination with a phototherapy blanket providing blue light 30 µW/cm/nm. During phototherapy extra fluids (10 ml/kg) were administered. Criteria for top-up transfusion: Hemoglobilin level <8 g/dL or <9.6 g/dL in the presence of clinical symptoms of anemia (such as lethargy, feeding problems, need for oxygen, or failure to thrive). * = not presented in a form usable for meta-analysis
Notes	Unpublished data and information supplied.

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer generated, sequence code kept by chief pharmacist.
Allocation concealment (selection bias)	Low risk	Pharmacy controlled block randomization.
Blinding of participants and personnel (performance bias)	Low risk	Identical coded drug boxes and vials. One infant's treatment unblinded due to serious adverse event. Unblinding unlikely to have affected study outcomes.
Blinding of outcome assessment (detection bias)	Low risk	Sequence code broken after three months follow-up period of last included patient.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	Low risk	Some outcomes are not completely reported as described in the published protocol. In protocol described that changes in bilirubin in the first 24 and 48 hours (%) will be measured. The paper only describes bilirubin levels at birth and maximum bilirubin during admission. In protocol described that top-up transfusions would be measured in the first week of life and after the first week until three months of life. The paper describes top-up transfusions for the whole period until three months after birth. However, data on changes in bilirubin levels are available and data on the top-up transfusion were provided for the first week and thereafter separately, therefore rated as low risk of bias.
Other bias	Low risk	One set of twins randomized to same treatment (done to avoid discrepant treatment for infants of same family). Re-analysis unlikely to change overall results.

Tanyer 2001[41]

Methods	Quasi-randomized trial
Participants	61 neonates with a positive direct Coombs' test; ABO or Rh or subgroup incompatibility, without "contributing risk factors (such as sepsis, drug use by mothers) that could raise bilirubin levels", not prematurely born and with bilirubin levels below ET criterion on admission.
Interventions	Treatment group 1: Single dose of IVIg 0.5 g/kg within 2-4 hours of admission (mean age of admission 2.3 days) plus phototherapy. (n = 20) Treatment group 2: IVIg 0.5 g/kg/day for 3 days within 2-4 hours of admission (mean age of admission 2 days) plus phototherapy. (n = 20) Control group: Phototherapy only (mean age of admission 2.8 days). (n = 21)
Outcomes	ETs, duration of phototherapy, and adverse events. Criteria for phototherapy: phototherapy was started once patient was admitted to the clinic and stopped when bilirubin level "decreased to the safe limit". Details phototherapy: performed using a white quartz halogen lamp (Air Shields Microlite Phototherapy system) with a distance between the infant and light source of 41 cm. Criteria for ET: performed when bilirubin levels exceeded the accepted limits which are shown in Table 1 or paper with reference to a study by Bryla et al.[61]

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	By order of admission. Given the distribution of bilirubin levels at admission, some infants may have been at ET thresholds on admission. This could have influenced treatment allocation.
Allocation concealment (selection bias)	High risk	Not concealed.
Blinding of participants and personnel (performance bias)	High risk	No placebo or other method of blinding described.
Blinding of outcome assessment (detection bias)	High risk	No placebo or other method of blinding described.
Incomplete outcome data (attrition bias)	Low risk	Complete data for all pre-defined outcomes.
Selective reporting (reporting bias)	High risk	Duration of hospitalization and top-up transfusions not reported.

Appendix 2: Details of excluded studies and studies awaiting assessment

Excluded studies

Girish 2008[44]

Reason for exclusion	This randomized controlled trial compared two doses of IVIg and had no placebo or "standard care" control group.

Spinelli 2001[45]

Reason for exclusion	This abstract only selectively reports outcome for enrolled infants who had moderate-severe hemolysis. Criteria for severity were not stated although stratification into mild, moderate and severe was pre-defined. Correspondence with authors seems unlikely to yield further information given interval since report, abstract only (no paper) and previous unsuccessful attempt.

Voto 1995[46]

Reason for exclusion	This randomized controlled trial compared a single dose of IVIg with control. However, none of the outcomes were reported in a form usable for the meta-analysis. Top-up transfusions and ET were not separately reported, bilirubin levels were presented as graphs rather than tables, and although the means of duration of phototherapy and hospitalization were presented in a table, the measure of variance was not clear. Correspondence with authors seems unlikely to yield further information given interval since report and previous unsuccessful attempt.

Studies awaiting assessment

Huang 2006[42]

Methods	Quasi-randomized trial. Randomization "by date of birth".
Participants	74 neonates who "tested positive for ABO HDN" and had "high bilirubin levels".
Interventions	Treatment group: IVIg 0.4-0.6 g/kg/day for 1-2 days plus albumin 1-2 g/kg/day for 2-3 days plus phototherapy. (n = 36) Control group: Phototherapy plus albumin 1-2 g/kg/day for 2-3 days. (n = 38)
Outcomes	ETs and duration of phototherapy.
Notes	Authors provided additional information, however it was still not clear what criteria for ET were used and consequently this study could not be included yet. Further information has been requested.

Wang 2002

Methods	RCT
Participants	121 neonates with: 1) blood type A or B of mothers with type O, 2) mothers with a Anti-A or Anti-B IgG >1:128, 3) a positive DAT or free-antibody test or antibody-release test, 4) methemoglobin reduction rate >75%, and 5) "clinical characteristics: hemolysis, jaundice, anemia and the like".
Interventions	Treatment group: IVIg 0.4 g/kg/day for 3 days (although in conclusion section 2-3 days) plus phototherapy and albumin 1 g/kg/day for 1-3 days and correcting acidosis, supplement of fluid and calories. (Mean (± SD) time of admission 41 ± 20 h). (n = 61) Control group: phototherapy and albumin 1 g/kg/day for 1-3 days and correcting acidosis, supplement of fluid and calories. (n = 51)
Outcomes	ETs, duration of phototherapy (not in form useable for meta-analysis), kernicterus, and adverse events.
Notes	Not stated whether any ethics approval or parental consent was given. Criteria for ET: not stated. Criteria for initiating phototherapy: not stated, (stopping criteria: transcutaneous bilirubin level <110mmol/L). Further information has been requested.

Appendix 3: Results of analyses

1. IVIg plus phototherapy versus phototherapy

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
1.1 Use of exchange transfusion (one or more)	14	942	RR (M-H, Random, 95% CI)	0.40 [0.27, 0.59]
1.2 Exchange transfusions per infant	12	788	MD (IV, Random, 95% CI)	-0.31 [-0.45, -0.17]
1.3 Use of top-up transfusion in 1st week	5	396	RR (M-H, Fixed, 95% CI)	1.05 [0.65, 1.69]
1.4 Top-up transfusions in 1st week per infant	4	280	MD (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
1.5 Use of top-up transfusion after 1st week	8	581	RR (M-H, Fixed, 95% CI)	1.20 [1.00, 1.45]
1.6 Top-up transfusions after 1st week per infant	4	316	MD (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]

1.7 Maximum serum bilirubin (µmol/L)	9	623	MD (IV, Fixed, 95% CI)	-17.52 [-25.20, -9.84]
1.8 Duration of phototherapy (days)	10	755	MD (IV, Random, 95% CI)	-0.87 [-1.24, -0.50]
1.9 Duration of hospitalization (days)	8	676	MD (IV, Fixed, 95% CI)	-1.50 [-1.72, -1.28]
1.10 Incidence of adverse reaction	13	837	RR (M-H, Fixed, 95% CI)	0.83 [0.43, 1.58]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

2. IVIg plus phototherapy versus phototherapy. Rh incompatibility only

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
2.1 Use of exchange transfusion (one or more)	9	426	RR (M-H, Fixed, 95% CI)	0.42 [0.29, 0.61]
2.2 Exchange transfusions per infant	9	426	MD (IV, Fixed, 95% CI)	-0.25 [-0.34, -0.16]
2.3 Use of top-up transfusion in 1st week	5	303	RR (M-H, Fixed, 95% CI)	1.08 [0.65, 1.77]
2.4 Top-up transfusions in 1st week per infant	4	280	MD (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
2.5 Use of top-up transfusion after 1st week	6	281	RR (M-H, Fixed, 95% CI)	1.09 [0.92, 1.28]
2.6 Top-up transfusions after 1st week per infant	3	204	MD (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]
2.7 Maximum serum bilirubin (µmol/L)	7	376	MD (IV, Fixed, 95% CI)	-21.40 [-30.43, -12.37]
2.8 Duration of phototherapy (days)	6	316	MD (IV, Fixed, 95% CI)	-1.21 [-1.40, -1.01]
2.9 Duration of hospitalization (days)	5	298	MD (IV, Fixed, 95% CI)	-1.47 [-1.75, -1.19]
2.10 Incidence of adverse reaction	8	403	RR (M-H, Fixed, 95% CI)	0.79 [0.22, 2.87]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

3. IVIg plus phototherapy versus phototherapy. ABO incompatibility only

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
3.1 Use of exchange transfusion (one or more)	4	229	RR (M-H, Fixed, 95% CI)	0.33 [0.18, 0.60]
3.2 Exchange transfusions per infant	4	229	MD (IV, Fixed, 95% CI)	-0.21 [-0.32, -0.10]
3.3 Use of top-up transfusion in 1st week	1	93	RR (M-H, Fixed, 95% CI)	0.80 [0.19, 3.38]
3.4 Use of top-up transfusion after 1st week	3	226	RR (M-H, Fixed, 95% CI)	5.02 [0.62, 40.67]
3.5 Maximum serum bilirubin (µmol/L)	1	93	MD (IV, Fixed, 95% CI)	-60.60 [-83.24, -37.96]
3.6 Duration of phototherapy (days)	3	249	MD (IV, Fixed, 95% CI)	-0.76 [-1.12, -0.41]
3.7 Duration of hospitalization (days)	3	249	MD (IV, Fixed, 95% CI)	-0.87 [-1.40, -0.35]
3.8 Incidence of adverse reaction	3	136	RR (M-H, Fixed, 95% CI)	1.00 [0.45, 2.21]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

4. IVIg plus phototherapy versus phototherapy. Gestational age ≥37 weeks

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
4.1 Use of exchange transfusion (one or more)	7	463	RR (M-H, Fixed, 95% CI)	0.37 [0.24, 0.57]
4.2 Exchange transfusions per infant	7	463	MD (IV, Fixed, 95% CI)	-0.18 [-0.25, -0.10]
4.3 Use of top-up transfusion in 1st week	4	296	RR (M-H, Fixed, 95% CI)	0.91 [0.48, 1.74]
4.4 Top-up transfusions in 1st week per infant	3	180	MD (IV, Fixed, 95% CI)	-0.01 [-0.35, 0.33]
4.5 Use of top-up transfusion after 1st week	4	240	RR (M-H, Fixed, 95% CI)	1.18 [0.81, 1.71]
4.6 Top-up transfusions after 1st week per infant	2	90	MD (IV, Fixed, 95% CI)	-0.03 [-0.20, 0.13]

4.7 Maximum serum bilirubin (µmol/L)	4	296	MD (IV, Fixed, 95% CI)	-26.81 [-35.97, -17.65]
4.8 Duration of phototherapy (days)	7	463	MD (IV, Fixed, 95% CI)	-1.01 [-1.17, -0.84]
4.9 Duration of hospitalization (days)	6	402	MD (IV, Fixed, 95% CI)	-1.20 [-1.45, -0.96]
4.10 Incidence of adverse reaction	7	463	RR (M-H, Fixed, 95% CI)	0.59 [0.09, 3.70]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

5. IVIg plus phototherapy versus phototherapy. Gestational age <37 weeks

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
5.1 Use of exchange transfusion (one or more)	2	82	RR (M-H, Fixed, 95% CI)	0.77 [0.31, 1.91]
5.2 Exchange transfusions per infant	2	82	MD (IV, Fixed, 95% CI)	-0.10 [-0.32, 0.12]
5.3 Use of top-up transfusion in 1st week	2	82	RR (M-H, Fixed, 95% CI)	1.39 [0.70, 2.73]
5.4 Top-up transfusions in 1st week per infant	2	82	MD (IV, Fixed, 95% CI)	0.08 [-0.12, 0.27]
5.5 Use of top-up transfusion after 1st week	2	82	RR (M-H, Fixed, 95% CI)	1.24 [0.93, 1.67]
5.6 Top-up transfusions after 1st week per infant	2	82	MD (IV, Fixed, 95% CI)	0.04 [-0.13, 0.20]
5.7 Maximum serum bilirubin (µmol/L)	2	82	MD (IV, Fixed, 95% CI)	-18.91 [-54.49, 16.68]
5.8 Duration of phototherapy (days)	2	82	MD (IV, Fixed, 95% CI)	-0.91 [-1.96, 0.14]
5.9 Duration of hospitalization (days)	2	82	MD (IV, Fixed, 95% CI)	-2.28 [-3.84, -0.72]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

6. IVIg plus phototherapy versus phototherapy. IVIg administration ≤12 hours after birth

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
6.1 Use of exchange transfusion (one or more)	7	427	RR (M-H, Fixed, 95% CI)	0.51 [0.35, 0.73]
6.2 Exchange transfusions per infant	6	353	MD (IV, Fixed, 95% CI)	-0.19 [-0.28, -0.10]
6.3 Use of top-up transfusion in 1st week	4	280	RR (M-H, Fixed, 95% CI)	1.18 [0.70, 2.00]
6.4 Top-up transfusions in 1st week per infant	4	280	MD (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
6.5 Use of top-up transfusion after 1st week	5	319	RR (M-H, Fixed, 95% CI)	1.10 [0.92, 1.31]
6.6 Top-up transfusions after 1st week per infant	3	204	MD (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]
6.7 Maximum serum bilirubin (µmol/L)	7	427	MD (IV, Fixed, 95% CI)	-16.99 [-25.86, -8.11]
6.8 Duration of phototherapy (days)	4	280	MD (IV, Fixed, 95% CI)	-1.22 [-1.42, -1.01]
6.9 Duration of hospitalization (days)	3	262	MD (IV, Fixed, 95% CI)	-1.46 [-1.76, -1.17]
6.10 Incidence of adverse reaction	7	427	RR (M-H, Fixed, 95% CI)	0.79 [0.22, 2.87]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

7. IVIg plus phototherapy versus phototherapy. IVIg administration >12 hours after birth

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
7.1 Use of exchange transfusion (one or more)	5	363	RR (M-H, Fixed, 95% CI)	0.27 [0.17, 0.44]
7.2 Exchange transfusions per infant	4	283	MD (IV, Fixed, 95% CI)	-0.29 [-0.41, -0.17]
7.3 Use of top-up transfusion in 1st week	1	116	RR (M-H, Fixed, 95% CI)	0.71 [0.24, 2.12]
7.4 Use of top-up transfusion after 1st week	2	150	RR (M-H, Fixed, 95% CI)	8.00 [1.03, 62.26]

7.5 Maximum serum bilirubin (µmol/L)	2	196	MD (IV, Fixed, 95% CI)	-19.10 [-34.41, -3.79]
7.6 Duration of phototherapy (days)	5	363	MD (IV, Fixed, 95% CI)	-0.74 [-1.00, -0.49]
7.7 Duration of hospitalization (days)	4	302	MD (IV, Fixed, 95% CI)	-1.60 [-1.93, -1.26]
7.8 Incidence of adverse reaction	4	283	RR (M-H, Fixed, 95% CI)	0.20 [0.01, 4.08]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

8. IVIg plus phototherapy versus phototherapy. Single dose of IVIg

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
8.1 Use of exchange transfusion (one or more)	10	724	RR (M-H, Fixed, 95% CI)	0.41 [0.29, 0.57]
8.2 Exchange transfusions per infant	9	675	MD (IV, Fixed, 95% CI)	-0.21 [-0.27, -0.14]
8.3 Use of top-up transfusion in 1st week	4	378	RR (M-H, Fixed, 95% CI)	1.05 [0.65, 1.69]
8.4 Top-up transfusions in 1st week per infant	3	262	MD (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
8.5 Use of top-up transfusion after 1st week	7	522	RR (M-H, Fixed, 95% CI)	1.19 [0.99, 1.42]
8.6 Top-up transfusions after 1st week per infant	4	316	MD (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]
8.7 Maximum serum bilirubin (µmol/L)	7	500	MD (IV, Fixed, 95% CI)	-22.68 [-31.17, -14.19]
8.8 Duration of phototherapy (days)	6	562	MD (IV, Fixed, 95% CI)	-0.95 [-1.12, -0.79]
8.9 Duration of hospitalization (days)	6	562	MD (IV, Fixed, 95% CI)	-1.21 [-1.46, -0.96]
8.10 Incidence of adverse reaction	10	724	RR (M-H, Fixed, 95% CI)	0.86 [0.43, 1.73]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

9. IVIg plus phototherapy versus phototherapy. Multiple doses of IVIg

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
9.1 Use of exchange transfusion (one or more)	3	98	RR (M-H, Fixed, 95% CI)	0.48 [0.27, 0.83]
9.2 Exchange transfusions per infant	2	52	MD (IV, Fixed, 95% CI)	-0.83 [-1.28, -0.38]
9.3 Use of top-up transfusion in 1st week	1	18	RR (M-H, Fixed, 95% CI)	Not estimable
9.4 Top-up transfusions in 1st week per infant	1	18	MD (IV, Fixed, 95% CI)	Not estimable
9.5 Use of top-up transfusion after 1st week	2	80	RR (M-H, Fixed, 95% CI)	2.09 [0.54, 8.13]
9.6 Maximum serum bilirubin (µmol/L)	2	64	MD (IV, Fixed, 95% CI)	22.89 [-12.00, 57.78]
9.7 Duration of phototherapy (days)	2	52	MD (IV, Fixed, 95% CI)	-1.00 [-1.90, -0.10]
9.8 Duration of hospitalization (days)	1	34	MD (IV, Fixed, 95% CI)	-1.41 [-2.51, -0.31]
9.9 Incidence of adverse reaction	3	98	RR (M-H, Fixed, 95% CI)	0.64 [0.11, 3.54]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

10. IVIg plus phototherapy versus phototherapy. High quality studies only.

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
10.1 Use of exchange transfusion (one or more)	2	172	RR (M-H, Fixed, 95% CI)	0.98 [0.48, 1.98]
10.2 Exchange transfusions per infant	2	172	MD (IV, Fixed, 95% CI)	-0.04 [-0.18, 0.10]
10.3 Use of top-up transfusion in 1st week	2	172	RR (M-H, Fixed, 95% CI)	1.18 [0.70, 2.00]
10.4 Top-up transfusions in 1st week per infant	2	172	MD (IV, Fixed, 95% CI)	0.05 [-0.07, 0.17]
10.5 Use of top-up transfusion after 1st week	2	172	RR (M-H, Fixed, 95% CI)	1.01 [0.80, 1.27]

10.6 Top-up transfusions after 1st week per infant	2	172	MD (IV, Fixed, 95% CI)	-0.00 [-0.12, 0.12]
10.7 Maximum serum bilirubin (µmol/L)	2	172	MD (IV, Fixed, 95% CI)	0.92 [-23.94, 25.79]
10.8 Duration of phototherapy (days)	2	172	MD (IV, Fixed, 95% CI)	-0.50 [-1.24, 0.24]
10.9 Duration of hospitalization (days)	2	172	MD (IV, Fixed, 95% CI)	-1.38 [-2.55, -0.20]
10.10 Incidence of adverse reaction	2	172	RR (M-H, Fixed, 95% CI)	2.86 [0.12, 68.10]

RR = Risk Ratio, M-H = Mantel-Haenszel, CI = Confidence Interval, MD = Mean Difference, IV = Inverse Variance

Part 3

Alloimmunizations other than Rh D

Chapter 7

Exchange transfusions and top-up transfusions in neonates with Kell hemolytic disease compared to Rh D hemolytic disease

Mirjam E.A. Rath
Vivianne E.H.J. Smits-Wintjens
Irene T.M. Lindenburg
Anneke Brand
Inge L. van Kamp
Dick Oepkes
Frans J. Walther
Enrico Lopriore

Vox Sang 2010; 100(3):312-316

Abstract

Objective

To evaluate neonatal outcome in Kell hemolytic disease compared to Rh D hemolytic disease.

Study design

Retrospective study of all (near)-term neonates with Kell (n = 34) and Rh D hemolytic disease (n = 157) admitted to our center between January 2000 and December 2008. We recorded the need for exchange transfusion and top-up transfusions up to three months of age.

Results

Neonates in the Kell group required less days of phototherapy than neonates in the Rh D group (2.4 versus 4.1 days, respectively (p = <0.01)). The percentage of neonates requiring an exchange transfusion was lower in the Kell group than in the Rh D group (6% (2/34) and 62% (98/157), respectively (p = <0.01)). The percentage of neonates in the Kell group and Rh D group requiring a top-up transfusion was 62% (21/34) and 72% (113/157), respectively (p = 0.20). The median number of top-up transfusions per neonate in the Kell group and Rh D group was 1 (interquartile range (IQR) 0-2) and 2 (IQR 0-2), respectively (p = 0.07).

Conclusion

Neonates with Kell hemolytic disease require less phototherapy and less exchange transfusions compared to neonates with Rh D hemolytic disease, but an equal number of top-up transfusions.

Introduction

Kell alloimmunization is second only to Rh D in causing antibody-mediated fetal anemia. Since the introduction of Rh D immunoprophylaxis, Kell antibodies account for 10% of antibody-mediated fetal anemia.[1] After introduction of routine antibody-screening of all pregnant women in the Netherlands in 1998, perinatal survival of fetuses with Kell hemolytic disease of the neonate (HDN) treated with intrauterine transfusion (IUT) increased from 61% to 100%.[2]

In contrast to Rh D HDN, fetal anemia in Kell HDN is often more severe due to concomitant suppression of erythropoiesis rather than hemolysis of erythrocytes.[3-5] Consequently, the immediate neonatal management in Kell HDN is different from Rh D HDN. A previous small study showed that neonates with Kell HDN have lower serum bilirubin levels and require less phototherapy and exchange transfusions (ETs) than neonates with Rh D hemolytic disease.[6]

In analogy with Rh D hemolytic disease, neonates with Kell HDN may require top-up transfusions for up to several months after birth.[7] Whether the incidence and severity of neonatal anemia in Kell hemolytic disease differs from neonates with Rh D hemolytic disease, is not known. Only a limited number of studies (mostly case reports) have been published on the severity of anemia in the postnatal period.[6,8-10]

The aim of this study was to evaluate neonatal and hematological outcome in a large series of neonates with Kell hemolytic disease compared to neonates with Rh D hemolytic disease.

Patients and Methods

All (near)-term neonates (gestational age ≥35 weeks) with hemolytic disease due to maternal Kell and Rh D alloimmunization, born between January 2000 and December 2008 at the Leiden University Medical Center (LUMC) were included in this retrospective observational study. Part of the neonates included in the Rh D group have previously been described in a different study.[11] Our center is the national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. We excluded all neonates with other types of HDN and neonates participating in an ongoing randomized trial for the use of immunoglobulin in Rh D hemolytic disease, which started in August 2006 at our institution (LIVIN-study: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=832).

The guideline for the application of phototherapy at our neonatal division has previously been described.[11,12] The guidelines for ET used at our neonatal division were changed

in December 2005. Before December 2005, criteria for ET included: (1) bilirubin level at birth >3.5 mg/dL (so-called early criterion) and/or (2) total serum bilirubin level above ET thresholds (rise of bilirubin value >0.5 mg/dL/hr despite intensive phototherapy). In neonates not treated with IUT, a hemoglobin level at birth of <12.9 g/dL was also considered as an early criterion for ET.[12] In December 2005 a new guideline of the American Academy of Pediatrics (AAP) with higher bilirubin thresholds for phototherapy and ET was implemented by our department.[13] The criteria for ET after December 2005 were: (1) total serum bilirubin above (higher) ET thresholds[13] and/or (2) rise of bilirubin >0.5 mg/dL/hr despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level. ET was performed with double-volume transfusion (160 mL/kg) using irradiated and leukocyte-depleted compatible erythrocytes. We recorded the following obstetric and neonatal data: fetal hemoglobin concentration at first IUT and number of IUTs, gestational age at birth, birth weight, hemoglobin level and reticulocyte count at birth, bilirubin level at birth, maximum bilirubin level during admission, duration of phototherapy (days), number of ETs required, number of top-up red blood cell transfusions received during the first 3 months of life and hemoglobin levels prior to the top-up transfusion. Data on the number of top-up transfusions and hemoglobin levels prior to the top-up transfusion in infants who received follow-up outside the LUMC were collected through correspondence with the local pediatrician or blood transfusion department.

After discharge from our center, top-up transfusions were performed in referring hospitals when hemoglobin levels were <8.0 g/dL, or <9.6 g/dL if clinical symptoms of neonatal anemia were present (such as lethargy, feeding problems, need for oxygen or failure to thrive). Folic acid (50 mcg/day) was administered orally during the first three months of life to all neonates with hemolytic disease.

Primary outcome was the number of ETs and the number of top-up transfusions. Outcome was compared between neonates with Kell hemolytic disease (Kell group) and neonates with Rh D hemolytic disease (Rh D group).

Data are reported as means and standard deviations (SD) or as medians and interquartile range (IQR), as appropriate. Statistical analysis was performed using Student-t-test and Mann-Whitney test for continuous variables. Chi square and Fisher's exact test were used for categorical variables, as appropriate. Linear regression analysis was performed using the Pearson Correlation coefficient. A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 16.0 (SPSS Inc, Chicago, IL, USA).

Results

During the study period 309 neonates with hemolytic disease were admitted to our neonatal nursery. Two hundred and seventy-seven (90%) of these neonates were born at a gestational age ≥35 weeks. Fifty-five neonates were excluded because of participation in a randomized trial for the use of intravenous immunoglobulin. Thirty-one neonates were excluded due to HDN caused by Rh c (n = 17), Rh C (n = 3), Rh E (n = 3), Cw (n = 2), Jka (n = 1), presence of both Rh D and Kell antibodies (n = 2), and unknown type of irregular antibody (n = 3). A total of 191 patients were included in this study, 34 (18%) in the Kell group and 157 (82%) in the Rh D group. Baseline characteristics in both groups are summarized in Table 1. Intrauterine transfusions (IUTs) were performed in 82% of neonates in the Kell group and 66% of neonates in the Rh D group (p = 0.07). The median number of IUTs in the Kell group and Rh D group was 3 (IQR 2-4, range 0-5) and 2 (IQR 0-4, range 0-6) respectively (p = 0.01). In the Kell group the median antibody titer at first IUT was 1:128 (range 1:2-8000).

Table 1. Baseline characteristics in neonates with Kell and Rh D hemolytic disease

	Kell (n = 34)	**Rh D** (n = 157)	**p-value**
Neonates treated with IUT, n (%)	28 (82)	104 (66)	0.07
Number of IUTs per neonate[a]	3 (2-4)	2 (0-3.5)	0.01
Gestational age at first IUT, weeks[a]	27 (23-29)	29 (24-33)	0.07
Hemoglobin level at first IUT, g/dL[a]	5.3 (3.5-7.3)	6.4 (5.0-7.4)	0.16
Gestational age at birth, weeks[a]	36 (36-37)	37 (36-37)	0.52
Birth weight, grams[b]	3190 ± 348	2947 ± 418	<0.01
Male, n (%)	25 (74)	92 (59)	0.11

[a] Value given as median (IQR), [b] Value given as mean ± SD

Phototherapy and ET

Detailed information on neonatal outcome in both groups, in particular treatment with phototherapy and ET is presented in Table 2. Mean bilirubin level at birth and maximum bilirubin level during admission were significantly lower in the Kell group than in the Rh D group, 3.1 versus 6.0 mg/dL (p = <0.01) and 8.0 versus 14.3 mg/dL (p = <0.01), respectively. Neonates in the Kell group required significantly less days of phototherapy than neonates in the Rh D group (2.4 and 4.1 mean days, respectively, (p = <0.01)). At least one ET was required in 6% (2/34) of the patients in the Kell group compared to 62% (98/157) of the

patients in the Rh D group (p = <0.01). The median number of ETs was 0 in the Kell group (IQR 0-0, range 0-1) and 1 in the Rh D group (IQR 0-1, range 0-5) (p = <0.01). None of the infants in the Kell group without IUT required an ET.

Table 2. Neonatal outcome in the Kell- and Rh D-group

	Kell (n = 34)	**Rh D** (n = 157)	**p-value**
Hemoglobin level at birth, g/dL[a]	7.9 ± 1.8	7.2 ± 1.6	0.01
Bilirubin level at birth, mg/dL[a]	3.1 ± 1.7	6.0 ± 2.3	<0.01
Reticulocyte count at birth, ‰[b,c]	12 (8-49)	21 (3-66)	0.90
Maximum bilirubin, mg/dL[b]	8.0 (3.9-10.7)	14.3 (10.8-16.9)	<0.01
Neonates treated with phototherapy, n (%)	31 (91)	154 (98)	0.07
Phototherapy, days[a,d]	2.4 ± 1.3	4.1 ± 1.7	<0.01
Neonates treated with ET, n (%)	2 (6)	98 (62)	<0.01
Number of ETs per neonate[b]	0 (0-0)	1 (0-1)	<0.01

[a] Value given as mean ± SD, [b] Value given as median (IQR), [c] Assessed in 15/34 and 81/157 neonates in the Kell and Rh D-group, respectively, [d] Assessed in 134/157 neonates with Rh D

Top-up transfusions

Complete information on the number of top-up red cell transfusions was obtained for 98% (188/191) of neonates. The percentage of neonates requiring a top-up transfusion was similar in the Kell group and Rh D group (62% (21/34) and 72% (113/157), respectively (p = 0.20). The median number of top-up transfusions per neonate in the Kell group and Rh D group was 1 (IQR 0-2, range 0-4) and 2 (IQR 0-2, range 0-6), respectively (p = 0.07). Mean hemoglobin level at first top-up transfusion and median number of days until first top-up transfusion were similar in both groups. Detailed information on the use of top-up transfusions in the Kell group and Rh D group is presented in Table 3.

In the sub-group analysis of neonates treated with IUT (n = 132), we found that neonates with Rh D HDN required significantly more top-up transfusions than neonates with Kell HDN (median of 2 range 0-6 and median of 1 range 0-4), respectively (p = 0.02). We performed a linear regression analysis between the number of IUTs and the reticulocyte count at birth in both groups. A higher number of IUTs was correlated with a lower reticulocyte count at birth in the Rh D group (Pearson Correlation coefficient -0.49; p = <0.001). This negative correlation was not found in the Kell group (Pearson Correlation coefficient -0.05; p = 0.85).

Table 3. Top-up transfusions in neonates with Kell and Rh D hemolytic disease

		Kell (n = 34)	Rh D (n = 157)	p-value
Neonates requiring top-up transfusions, n (%)		21 (62)	113 (72)	0.20
Number of top-up transfusions per neonate[a]		1 (0-2)	2 (0-2)	0.07
Neonates requiring:	1 top-up transfusion, n (%)	10 (48)	39 (35)	0.62
	2 top-up transfusions, n (%)	8 (38)	40 (35)	0.77
	3 top-up transfusions, n (%)	2 (9)	16 (14)	0.54
	4 top-up transfusions, n (%)	1 (5)	14 (12)	0.31
	5 top-up transfusions, n (%)	0 (0)	2 (2)	1.00
	6 top-up transfusions, n (%)	0 (0)	1 (1)	1.00
Days after birth until first top-up transfusion[a]		16 (1-31.5)	17.5 (1-33.5)	0.56
Hemoglobin level at first top-up transfusion, g/dL[b]		8.2 ± 1.4	8.4 ± 1.5	0.73
Number of top-up transfusions (per neonate) in the subgroup treated with IUT[a]		1 (0-2)	1.9 (1-3)	0.02

[a] Value given as median (IQR), [b] Value given as mean ± SD

Discussion

This study shows that fetuses with severe Kell hemolytic disease are more often treated with IUT compared to fetuses with Rh D hemolytic disease. Subsequently, infants with HDN due to Kell-antibodies need less phototherapy and ETs in the neonatal period than neonates with Rh D hemolytic disease. However, the need for top-up transfusions was similar in both groups.

Various researchers have suggested that anemia in Kell hemolytic disease is caused primarily by erythroid suppression rather than hemolysis, as in Rh disease.[3,4] An alternative theory is that anti-Kell-antibodies are responsible for the destruction of early erythroid progenitor cells, which lack hemoglobin.[5,14] A lower amniotic fluid bilirubin and only mild neonatal hyperbilirubinemia in Kell hemolytic disease compared to Rh D are consistent with this theory. In addition, an in vitro study by Vaughan et al. demonstrated that human monoclonal anti-Kell antibodies and the serum of women with anti-Kell antibodies specifically inhibit the growth of Kell-positive erythroid progenitor cells.[5] Vaughan et al. found no correlation between the anti-Kell antibody titer and the degree of inhibition.[5] Poor correlation between antibody titer and disease severity in Kell supports the theory that Kell alloimmunization has a different pathogenesis than Rh alloimmunization. In a recent study of 43 pregnancies with Kell alloimmunization, we found that the vast majority of severely

affected cases had antibody titers of 1:32 or more. Nevertheless, to be on the safe side, we recommended that all pregnancies with Kell titers of 1:2 or higher (and a proven Kell positive fetus) should be closely monitored.[2]

In accordance with previous studies from the group of Weiner[4] and our group[15] we found that antenatal course of fetuses with Kell alloimmunization is different from Rh D hemolytic disease. Fetuses with Kell alloimmunization have lower hemoglobin levels at first IUT and require more IUTs. Moreover, the first IUT was performed at an earlier gestational age than in Rh D fetuses.[15] These findings underscore that fetal anemia is more severe in Kell sensitized fetuses than in Rh D sensitized fetuses. Weiner et al. also found a significant lower reticulocyte count, reflecting the destruction of Kell expressing erythroid progenitor cells in Kell hemolytic disease.[4]

In terms of neonatal management and outcome, this study shows that neonates with Kell hemolytic disease have milder hyperbilirubinemia, requiring less phototherapy and ETs than infants with Rh D hemolytic disease. In our study we found no relation between lack of IUT and number of ETs or top-up transfusions. Our findings are consistent with previous reports[6,8,16-18] and reflect the observation that hemolysis of mature (hemoglobinized) erythrocytes in Kell hemolytic disease is less than in Rh D hemolytic disease.[3,4]

Given the significantly higher number of IUTs in the Kell group, one could expect an increased incidence of postnatal anemia (and top-up transfusions). As shown in previous studies, repeated IUTs result in a decreased reticulocyte count, indicating a suppression of fetal erythropoiesis.[12,19] In contrast, we found a trend towards less top-up transfusions in neonates with Kell hemolytic disease compared to neonates with Rh D hemolytic disease, however this difference was not significant ($p = 0.07$). This finding could support the fact that fetal and neonatal anemia due to Kell alloimmunization has a different pathogenesis than Rh alloimmunization. Larger studies are required to confirm these findings. In contrast to anti-D, most anti-Kell antibodies have a strong lytic potential which also affects red cell precursor cells.[3,4,19] Vaughan and colleagues found no correlation between the antibody titer and the degree of inhibition of Kell-positive erythroid progenitor cells.[5] The titers of anti-Kell antibodies associated with fetal anemia are generally substantially lower compared to ten to 100 fold higher titers in case of Rh D hemolytic disease. Consequently anti-D antibodies may circulate in the newborn for a longer period after birth, whereas Kell antibodies disappear sooner, which may explain the more moderate late anemia in Kell hemolytic disease, despite concomitant suppression of the erythropoiesis.[5]

This is the first study comparing the degree of postnatal anemia in a relatively large number of infants with Kell- and Rh D hemolytic disease. Santiago et al. described three neonates with Kell HDN of whom only one required a top-up transfusion.[17]

Collinet et al. reported a case of severe fetal anemia due to Kell alloimmunization, which was postnatally treated with two top-up transfusions and recombinant erythropoietin and iron supplementation.[7]

The results of this study should be interpreted with care due to the small number of neonates with Kell hemolytic disease in this study which is inherent to the low incidence of this disease. Larger, multicenter studies are required to confirm our findings.

In conclusion, although neonates with Kell hemolytic disease require less phototherapy and exchange transfusions, the equal need for top-up transfusions justifies similar follow-up management as in Rh D hemolytic disease. Finally, because of the destruction of red cell precursor cells as well, treatment with erythropoietin may be more effective in neonates with Kell hemolytic disease than in neonates with Rh D hemolytic disease.[9,10]

References

1. Lee S, Russo D, Redman CM: The Kell blood group system: Kell and XK membrane proteins. Semin Hematol 2000; 37(2):113-121.
2. Kamphuis MM, Lindenburg I, Van Kamp IL, Meerman RH, Kanhai HH, Oepkes D: Implementation of routine screening for Kell antibodies: does it improve perinatal survival? Transfusion 2008; 48(5):953-957.
3. 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.
4. Weiner CP, Widness JA: Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anemia. Am J Obstet Gynecol 1996; 174(2):547-551.
5. 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.
6. Babinszki A, Lapinski RH, Berkowitz RL: Prognostic factors and management in pregnancies complicated with severe kell alloimmunization: experiences of the last 13 years. Am J Perinatol 1998; 15(12):695-701.
7. Collinet P, Subtil D, Puech F, Vaast P: Successful treatment of extremely severe fetal anemia due to Kell alloimmunization. Obstet Gynecol 2002; 100(5 Pt 2):1102-1105.
8. Wenk RE, Goldstein P, Felix JK: Kell alloimmunization, hemolytic disease of the newborn, and perinatal management. Obstet Gynecol 1985; 66(4):473-476.
9. Dhodapkar KM, Blei F: Treatment of hemolytic disease of the newborn caused by anti-Kell antibody with recombinant erythropoietin. J Pediatr Hematol Oncol 2001; 23(1):69-70.
10. Manoura A, Korakaki E, Hatzidaki E, Saitakis E, Maraka S, Papamastoraki I, Matalliotakis E, Foundouli K, Giannakopoulou C: Use of recombinant erythropoietin for the management of severe hemolytic disease of the newborn of a K0 phenotype mother. Pediatr Hematol Oncol 2007; 24(1):69-73.
11. Rath ME, Smits-Wintjens VE, 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(1):65-70.
12. 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.
13. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation: Pediatrics 2004; 114(1):297-316.
14. Daniels G, Hadley A, Green CA: Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion 2003; 43(1):115-116.
15. 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.
16. Bowman JM, Pollock JM, Manning FA, Harman CR, Menticoglou S: Maternal Kell blood group alloimmunization. Obstet Gynecol 1992; 79(2):239-244.
17. Santiago JC, Ramos-Corp, Oyonarte S, Montoya F: Current clinical management of anti-Kell alloimmunization in pregnancy. Eur J Obstet Gynecol Reprod Biol 2008; 136(2):151-154.

18. McKenna DS, Nagaraja HN, O'Shaughnessy R: Management of pregnancies complicated by anti-Kell isoimmunization. Obstet Gynecol 1999; 93(5 Pt 1):667-673.
19. 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.

Chapter 8

Postnatal outcome in neonates with severe Rhesus c compared to Rhesus D hemolytic disease

Mirjam E.A. Rath
Vivianne E.H.J. Smits-Wintjens
Irene T.M. Lindenburg
Claudia C. Folman
Anneke Brand
Inge L. van Kamp
Dick Oepkes
Frans J. Walther
Enrico Lopriore

Transfusion; in press

Abstract

Background

Neonates with Rhesus c (Rh c) hemolytic disease of the fetus and newborn (HDFN) are often managed in the same way as neonates with Rhesus D (Rh D) HDFN, although evidence to support this policy is limited. The objective of this study was to evaluate neonatal outcome in severe Rh c HDFN compared to Rh D HDFN.

Methods

Retrospective study of (near-)term neonates with severe Rh c (n = 22) and Rh D HDFN (n = 103) (without additional antibodies) admitted to the Leiden University Medical Center between January 2000 and October 2011. The need for intrauterine transfusions (IUT), phototherapy, exchange transfusions (ET) and top-up transfusions up to three months of age were recorded and compared between both groups.

Results

Although there was a trend for a slightly more severe antenatal course of Rh D HDFN reflected by an earlier need for and higher number of IUTs (median (IQR) 2 (1.5-4) versus 2 (1-2) in Rh c HDFN, p = 0.070), no significant differences were found for the postnatal course between Rh c and Rh D group in days of phototherapy (mean days 4.8 and 4.6, respectively (p = 0.569)), need for ET (50% versus 44%, respectively (p = 0.589)) and top-up transfusions (62% versus 78%, respectively (p = 0.128)).

Conclusion

Postnatal outcome in neonates with severe Rh c HDFN is similar compared to neonates with severe Rh D hemolytic disease in terms of days of phototherapy, need for ET and need for top-up transfusions. These results justify a similar postnatal management of neonates with Rh D and Rh c HDFN.

Introduction

Anti-c is, after anti-D and anti-Kell, the most important red cell antibody causing severe hemolytic disease of the fetus and newborn (HDFN) necessitating intrauterine red cell transfusions (IUTs).[1]

The postnatal outcome in neonates with Rhesus c (Rh c) HDFN is not well known. Neonatologists manage neonates with Rh c HDFN in the same way as they manage neonates with Rhesus D (Rh D) HDFN. However, this policy is based on only a few case reports and case series with limited information. No studies have been published comparing outcome in Rh c and Rh D HDFN with respect to need for IUTs, the incidence and severity of neonatal anemia, hyperbilirubinemia, phototherapy, number of exchange transfusions (ETs) and top-up transfusions.

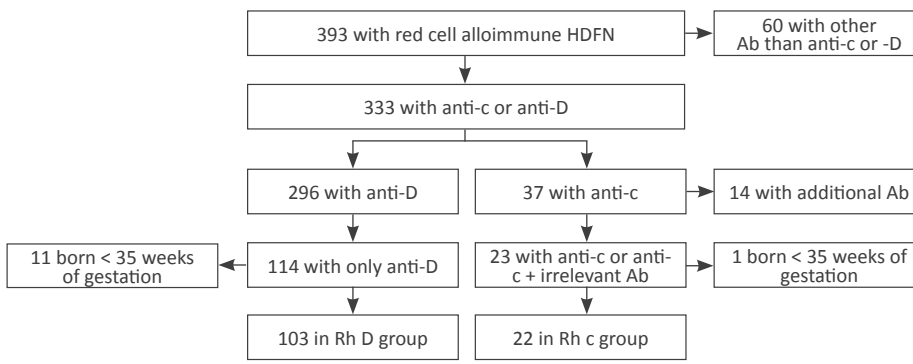
The objective of this study was to evaluate postnatal outcome and management in a large series of neonates with severe Rh c HDFN compared to neonates with Rh D HDFN. In addition, we investigated whether anti-c as compared to anti-D caused prolonged anemia needing late top-up transfusions in relation to antibody titers at birth.

Patients and Methods

Neonates with hemolytic disease due to maternal Rh c and Rh D alloimmunization, born between January 2000 and October 2011 at the Leiden University Medical Center (LUMC) were eligible for this retrospective observational study. Since neonatal hemolytic disease can be more severe in the presence of multiple antibodies directed against fetal antigens,[1] neonates with other clinically relevant antibodies directed against fetal antigens in addition to anti-c or anti-D were excluded as well as neonates born <35 weeks' gestation (Figure 1). Neonates with additional maternal antibodies, for which the child is lacking the antigen, were not excluded in the Rh c group. Table 1 provides a complete overview of all antibody combinations in Rh c and Rh D alloimmunization to outline the real clinical problem. Antibody titers were determined in the fetal period (maternal titers) and at birth by serial dilution in standard serological techniques using indirect antiglobulin test.[2,3] Functional potential of anti-D and anti-c antibodies were evaluated by the Antibody Dependent Cellular Cytotoxicity (ADCC) assay. This functional bioassay estimates the lytic potential of the antibodies in vitro aiming to predict severity of HDFN. The percentage of hemolysis in ADCC has been validated for Rh D.[4,5] In case of ADCC ≥30% and/or titer ≥1:16 patients are generally referred for ultrasound and Doppler assessment of the peak velocity in the middle cerebral artery (MCA) to determine the need for IUT.[6] The LUMC is the national

referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. A subgroup of the neonates has previously been described in different studies.[7-11]

Figure 1. Diagram of inclusion of neonates for Rh c versus Rh D study



Ab = antibody
Irrelevant Ab = Ab for which the child is lacking the antigen

IUT was scheduled when the peak velocity in the MCA was ≥1.50 multiples of the median. In all cases, blood was transfused, although occasionally, the degree of fetal anemia was only mild. Our IUT treatment protocol for Rh D and Rh c alloimmunization is identical. In December 2005 new guidelines for phototherapy and ET based on the guidelines of the American Academy of Pediatrics were implemented in our clinic. Intensive phototherapy (= 30µW/cm2/nm) using 4 lamps including a phototherapy blanket was started as soon as the infant was admitted and discontinued when bilirubin level was below phototherapy thresholds.[9] Criteria for ET before December 2005 included: (1) bilirubin level at birth >3.5 mg/dL (so-called early criterion) and/or (2) total serum bilirubin level above ET thresholds (and rise of bilirubin value >0.5 mg/dL/h despite intensive phototherapy).[9] In neonates not treated with IUT, a hemoglobin level at birth of <12.9 g/dL was also considered as an early criterion for ET. The criteria for ET after December 2005 were: (1) total serum bilirubin above (higher) ET thresholds and/or (2) rise of bilirubin >0.5 mg/dL/h despite intensive phototherapy, and/or (3) clinical symptoms of acute bilirubin encephalopathy regardless of bilirubin level.[9] ET was performed with double-volume transfusion (160 mL/kg) using irradiated and leukocyte-depleted erythrocytes compatible with mother and child. Top-up transfusions were performed in our clinic and after discharge in referring hospitals when hemoglobin levels were <8.0 g/dL, or <9.6 g/dL if clinical symptoms of anemia were present

(lethargy, feeding problems, need for oxygen or failure to thrive). Our phototherapy, ET and top-up transfusion guidelines for neonates with Rh D and Rh c HDFN are identical. Folic acid (50 mcg/day) was administered orally during the first three months of life to all neonates with hemolytic disease.

Table 1. Specificity of all antibody combinations in cases with anti-D and anti-c

Cases with anti-c (n = 37)		**Cases with anti-D** (n = 296)	
Antibody specificity	**Frequency**	**Antibody specificity**	**Frequency**
c	17	D	128
c, E	5	D, C	108
c, Kell	2	D, C, E	12
c, E, Jk^a	1	D, E	10
c, Kell, Jk^a, M	1	D, C, Jk^a	6
c, Fy^a	1	D, Jk^a	5
c, Jk^a	1	D, C, Fy^a	4
c, $Lewis^a$	1	D, Kell	2
c, C^w	1	D, C, M	2
E, c	5	D, C, Kp^a	2
Kell, c	1	D, E, C	2
Fy^a, c	1	D, Fy^a	1
		D, C, G	1
		D, C, Jk^b	1
		D, C, Kell	1
		D, C, Fy^b	1
		D, E, Fy^a	1
		D, E, Jk^a	1
		D, Kell, C	1
		D, Kell, E	1
		D, Fy^a, C	1
		D, Jk^a, C	1
		D, Jk^a, S	1
		C, D	1
Antibodies were placed in order based on highest antibody titer.		E, D	1
A possible fourth antibody was not recorded.		Fy^a, C, D	1

We recorded the following obstetric and neonatal data: fetal hemoglobin concentration at first IUT and number of IUTs, maximum maternal antibody titer, gestational age at birth, birth weight, born before/after December 2005 (implementation new ET guideline), hemoglobin level and reticulocyte count at birth, titer of irregular antibody test at birth, bilirubin level at birth, maximum bilirubin level during admission, duration of phototherapy, number of ETs required, number of top-up red blood cell transfusions received during the first three months of life and hemoglobin levels prior to the top-up transfusions. Data on the number of top-up transfusions and hemoglobin levels prior to the top-up transfusions in infants who received follow-up outside the LUMC were collected through correspondence with the local pediatrician or blood transfusion department.
Primary outcome was the number of ETs and top-up transfusions. Outcome was compared between neonates with Rh c HDFN (Rh c group) and neonates with Rh D HDFN (Rh D group).
Data are reported as means with standard deviation (SD) or as medians with interquartile range (IQR), as appropriate. Statistical analysis was performed using Student-t-test and Mann-Whitney test for continuous variables. Chi square and Fisher's exact test were used for categorical variables, as appropriate. To assess the relationship between antibody titers and transfusion requirements Spearman correlations were calculated. A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 17.0 (SPSS Inc, Chicago, IL, USA).

Results

During the study period 393 neonates with hemolytic disease due to red cell alloimmunization were admitted to our neonatal nursery. Figure 1 shows the derivation of the included population and the reasons for exclusion. A total of 125 patients were included in this study, 22 (18%) in the Rh c group and 103 (82%) in the Rh D group. Baseline characteristics in both groups are summarized in Table 2. Intrauterine transfusions (IUTs) were performed in 41% of neonates in the Rh c group and 59% of neonates in the Rh D group (p = 0.116). The median number of IUTs in the Rh c group and Rh D group was 2 (IQR 1-2, range 1-3) and 2 (IQR 1.5-4, range 1-6), respectively (p = 0.070). The maximum ADCC result was significantly lower in the Rhesus c group (median 45%, range <10% - >80%) compared to the Rhesus D group (median >80%, range <10% - >80%) (p = <0.001).

Table 2. Baseline characteristics of Rh c group and Rh D group

	Rh c group (n = 22)	**Rh D group** (n = 103)	**p-value**
Neonates treated with IUT, n (%)	9 (41)	61 (59)	0.116
Number of IUTs in IUT treated neonates[a]	2 (1-2)	2 (1.5-4)	0.070
Gestational age at first IUT, weeks[a]	33 (30-34)	29 (25-33)	0.127
Hemoglobin level at first IUT, g/dL[b,c]	8.5 ± 1.8	6.5 ± 2.3	0.015
Maximum ADCC test result, %[a,d]	45 (11.25-80)	>80 (65 - >80)	<0.001
Maximum maternal antibody titer,[a,e]	1:1000 (1:1000-1:3500)	1:4000 (1:1000-1:8000)	0.001
Titer of irregular antibody test at birth in cord blood[a,f]	1:200 (1:128-1:500)	1:500 (1:64-1:2000)	0.087
Gestational age at birth, weeks[a]	37 (36-38)	37 (36-37)	0.087
Birth weight, kg[b]	3.21 ± 0.45	3.00 ± 0.42	0.107
Male, n (%)	14 (64)	65 (63)	0.963
Hydrops at birth, n (%)	0 (0)	2 (2)	1.000
Born before ET guideline change in December 2005, n (%)	13 (59)	50 (49)	0.391

[a] Value given as median (IQR), [b] Value given as mean ± SD, [c] Assessed in 59/61 Rh D neonates treated with IUT, [d] Assessed in 16/22 in Rhesus c group and 97/103 in Rhesus D group, [e] Assessed in 20/22 in Rhesus c group and 97/103 in Rhesus D group, [f] Assessed in 15/22 in Rhesus c group and 55/103 in Rhesus D group

Blood results at birth, phototherapy and ET

Detailed information on neonatal outcome in both groups, in particular treatment with phototherapy and ET is presented in Table 3. Although reticulocyte count at birth was only measured in 55% (12/22) and 63% (65/103) of neonates of the Rh c and Rh D group, respectively, the median absolute reticulocyte count of neonates with Rh c HDFN was higher than of neonates with Rh D HDFN: 25.4 (IQR 8.9-49.2) and 15.1 (IQR 1.6-29.2), respectively, p = 0.100). There were no significant differences in bilirubin levels, phototherapy treatment and ETs between both groups.

Top-up transfusions

Complete information on the number of top-up red cell transfusions was obtained for 98% (123/125) of neonates. We found no significant differences between the percentage of neonates requiring at least one top-up transfusion, the median number of top-up transfusions per neonate and the median number of days after birth until the last top-up transfusion between the two groups. Detailed information on the use of top-up transfusions in the Rh c group and Rh D group is presented in Table 4.

Table 3. Neonatal outcome and management in Rh c group and Rh D group

	Rh c group (n = 22)	Rh D group (n = 103)	p-value
Hemoglobin level at birth, g/dL[a]	12.4 ± 2.7	12.3 ± 2.9	0.599
Bilirubin level at birth, mg/dL[a]	6.9 ± 4.7	6.2 ± 3.0	0.990
Reticulocyte percentage at birth, %[b,c]	9 (4.2-15.2)	5.8 (0.8-8.9)	0.035
Absolute reticulocyte count at birth, 10^9/L[b,c]	25.4 (8.9-49.2)	15.1 (1.6-29.2)	0.100
Maximum bilirubin, mg/dL[a,d]	15.9 ± 4.3	14.7 ± 4.7	0.136
Neonates treated with phototherapy, n (%)	21 (95)	101 (98)	0.443
Phototherapy, days[a,e]	4.8 ± 2.4	4.6 ± 1.8	0.569
Neonates treated with ET, n (%)	11 (50)	55 (44)	0.589
Number of ETs per neonate[b]	2 (1-2)	1 (1-2)	0.076

[a] Value given as mean ± SD, [b] Value given as median (IQR), [c] Assessed in 12/22 in Rhesus c group and 65/103 in Rhesus D group, [d] Assessed in 21/22 in Rhesus c group, [e] Assessed in 93/103 in Rhesus D group

Table 4. Top-up transfusions in neonates in Rh c group and Rh D group

		Rh c group (n = 22)	Rh D group (n = 103)	p-value
Neonates requiring top-up transfusions, n (%)		13/21 (62)	80/103 (78)	0.128
Number of top-up transfusions per neonate[a,b]		2 (1-2)	2 (1-3)	0.418
Neonates requiring:	1 top-up transfusion, n (%)	5 (42)	28 (35)	
	2 top-up transfusions, n (%)	5 (42)	25 (32)	
	3 top-up transfusions, n (%)	1 (8)	10 (13)	
	4 top-up transfusions, n (%)	0 (0)	11 (14)	
	5 top-up transfusions, n (%)	0 (0)	2 (3)	
	6 top-up transfusions, n (%)	1 (8)	3 (4)	
Days after birth until first top-up transfusion[a,c]		19 (1-28.5)	10 (1.5-30.5)	0.972
Days after birth until last top-up transfusion[a,d]		31.5 (11-55)	42 (27-58)	0.337
Hemoglobin level at first top-up transfusion, g/dL[e,f]		8.26 ± 1.53	8.21 ± 1.47	0.900
Number of top-up transfusions per neonate in subgroup treated with IUT[a,g]		1 (1-2)	2 (2-4)	0.040
Days after birth until last top-up transfusion in subgroup treated with IUT[a]		23 (13.5-50.5)	48 (32.3-62.8)	0.092

[a] Value given as median (IQR), [b] Assessed in 12/13 in Rh c group and 79/80 in Rh D group, [c] Assessed in 77/80 in Rh D group, [d] Assessed in 12/13 in Rh c group and 75/80 in Rh D group , [e] Value given as mean ± SD, [f] Assessed in 75/80 in Rh D group, [g] Only assessed in neonates treated with top-up transfusions

In the sub-group analysis of neonates treated with IUT (n = 70), we found a significantly higher number of top-up transfusions in the Rh D group compared to the Rh c group (median (IQR) 2 (2-4) versus 1 (1-2), respectively, p = 0.040).

Antibody titer at birth and maximum maternal antibody titer in relation to postnatal transfusions

Antibody titer at birth (measured in cord blood) was assessed in 15/22 neonates in the Rhesus c group and 55/103 neonates in the Rhesus D group.

In the Rhesus c group, we found a positive correlation between antibody titer at birth and need for ET (correlation coefficient 0.614; p = 0.015). In case an ET was indicated in the Rh c group, antibody titer at birth was never <1:200. Antibody titer at birth is not correlated to the number of ETs, the need for top-up transfusions and the number of top-up transfusions in the Rh c group.

In the Rhesus D group, we found a positive correlation between antibody titer and need for top-up transfusions and number of top-up transfusions (correlation coefficients 0.406, p = 0.002 and 0.579, p = <0.001, respectively). Antibody titer is not correlated to the need for ET or the number of ETs in the Rh D group.

Maximum antibody titer was assessed in the mothers of 97/103 and 20/22 neonates of the Rh D and Rh c group respectively. Maximum maternal antibody titer is correlated to need for and number of top-up transfusions in the Rh D group (correlations coefficients 0.277, p = 0.006 and 0.537, p = <0.001, respectively), but not in the Rh c group. Maximum maternal antibody titer was not correlated to the need for ET or the number of ETs in the Rh D and Rh c group.

Discussion

This study shows that the need for phototherapy, ETs and top-up transfusions is similar between neonates with severe Rh c and Rh D HDFN. In particular number and timing of top-up transfusions were similar between both groups.

This is the first large study describing the postnatal outcome and management in neonates with severe Rh c HDFN compared to neonates with Rh D HDFN. Several cohort studies have been published on anti-c immunization in pregnancy, but postnatal management in Rh c alloimmunization was extrapolated from the data available for the management of Rh D HDFN.

We found a trend towards a lower number of IUTs in neonates with Rh c HDFN compared to Rh D HDFN suggesting that fetuses with Rh c HDFN may have less severe hemolysis.

The significantly lower ADCC test and maximum maternal antibody titer results in the Rh c group are consistent with this trend. One previous small study reported a median number of 3 IUTs in 57 affected pregnancies with Rh D alloimmunization and the number of IUTs in 2 pregnancies with Rh c alloimmunization (2 and 3 IUTs), but the study was to small to show any differences.[12] In a study from Hackney et al., 17% (8/46) of fetuses with Rh c HDFN required treatment with IUT. The mean number of IUTs in their study was 5 (range 2-9).[13] Kozlowski et al. reported that only 1 of 14 infants with Rh c HDFN who required ET received three IUTs.[14] A prospective study was performed in the Netherlands between 2003 and 2005, including 118 pregnant women with anti-c as dominant antibody. Only one fetus required an IUT, 6 received no IUT but one or more ETs (in some cases followed by top-up transfusion) and 5 received only top-up transfusion(s).[15] The high percentage of neonates with Rh c HDFN treated with IUT in our study reflects the more severely diseased group of neonates because our center is the national referral center for HDFN. Although our IUT-protocol is similar for Rh D as for Rh c HDFN, the hemoglobin level before first IUT was significantly higher in fetuses with Rh c HDFN compared to fetuses with Rh D HDFN. In addition, the timing of first IUT in neonates with Rh c is later (median of 2.6 weeks) compared to neonates with Rh D. This influences the hemoglobin level before first IUT since fetal hemoglobin level increases with higher gestational age.[16]

At birth, neonates with Rh c HDFN showed a significantly higher reticulocyte percentage and a trend towards a lower absolute reticulocyte count compared to neonates with Rh D HDFN in this study. This might be due to the lesser amount of IUTs in the Rh c group since IUT suppresses erythropoiesis.[17] In addition, in case of a high titer, anti-D antibodies can hemolyse reticulocytes and cause prolonged anemia.[18,19]

Treatment with phototherapy and ET was not significantly different between both groups. Our percentages of neonates with Rh c HDFN requiring phototherapy (96%) and ET (50%) are not consistent with previous studies. Most studies report lower rates of phototherapy (range: 7 to 77%) and ET (range: 5 to 17%).[13-15,20-22] In the most recent study of Karagol et al. 77% (17/22) of neonates with anti-c HDFN required phototherapy and 9% (2/22) required ET.[23] As mentioned before, these figures cannot be compared between studies because of different patient populations and our cohort represents a population of more severely diseased neonates.

Postnatal anemia requiring top-up transfusion and time until the last top-up transfusion were also not significantly different between neonates with Rh c and Rh D HFDN. Nevertheless, we did find a trend towards a lower number of top-up transfusions in the sub-group of neonates with Rh c HDFN treated with IUT compared to Rh D HDFN.

The positive correlation between antibody titer (measured in cord blood) and top-up

transfusions in our Rh D group is in accordance with other studies showing that (maternal) anti-D titer is a good indicator of the severity of hemolytic disease.[24] For Rh c HDFN Hackney et al. showed that all neonates who required IUT or with a hemoglobin level of <10 g/dL at birth had an antibody titer of ≥1:32.[13] The predictive value of antibody titer on postnatal transfusion requirements in Rh c HDFN has not been evaluated yet. However, further studies are needed to confirm the positive correlation between antibody titer and the need for ET in Rh c HDFN in our study.

The results of this study should be interpreted with care due to the relatively small number of neonates with severe Rh c HDFN which is inherent to the low incidence of this disease. In addition, some results were also limited by missing data of outcome variables.

In conclusion, severe Rh c HDFN behaves postnatally similar as Rh D HDFN in terms of need of phototherapy, ETs and top-up transfusions and justifies similar follow-up management. As suggested for Rh D HDFN, also for Rh c, the titer at birth might be helpful to predict the postnatal outcome.

References

1. Spong CY, Porter AE, Queenan JT. Management of isoimmunization in the presence of multiple maternal antibodies. Am.J.Obstet.Gynecol. 2001 Aug;185(2):481-4.
2. Guidelines for blood grouping and red cell antibody testing during pregnancy. British Committee for Standards in Haematology, Blood Transfusion Task Force. Transfus.Med. 1996 Mar;6(1):71-4.
3. ACOG Practice Bulletin No. 75: Management of alloimmunization during pregnancy. Obstet. Gynecol. 2006 Aug;108(2):457-64.
4. Engelfriet CP, Ouwehand WH. ADCC and other cellular bioassays for predicting the clinical significance of red cell alloantibodies. Baillieres Clin.Haematol. 1990 Apr;3(2):321-37.
5. Engelfriet CP, Overbeeke MA, Dooren MC, Ouwehand WH, von dem Borne AE. Bioassays to determine the clinical significance of red cell alloantibodies based on Fc receptor-induced destruction of red cells sensitized by IgG. Transfusion 1994 Jul;34(7):617-26.
6. 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 Aug;83(8):731-7.
7. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, Te Pas AB, Kramer CM, Oepkes D, Brand A, Lopriore E. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011 Apr;127(4):680-6.
8. Smits-Wintjens VE, 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 Feb 18;101(4):306-10.
9. Rath ME, Smits-Wintjens VE, 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 Mar 15;99(1):65-70.
10. Rath ME, Smits-Wintjens VE, Lindenburg IT, Brand A, Van Kamp IL, Oepkes D, Walther FJ, Lopriore E. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang. 2011 Apr;100(3):312-6.
11. Rath ME, Smits-Wintjens VE, Oepkes D, van Zwet EW, Van Kamp IL, Brand A, Walther FJ, Lopriore E. Thrombocytopenia at birth in neonates with red cell alloimmune haemolytic disease. Vox Sang. 2012 Apr;102(3):228-33.
12. Somerset DA, Moore A, Whittle MJ, Martin W, Kilby MD. An audit of outcome in intravascular transfusions using the intrahepatic portion of the fetal umbilical vein compared to cordocentesis. Fetal Diagn.Ther. 2006;21(3):272-6.
13. Hackney DN, Knudtson EJ, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-c isoimmunization. Obstet.Gynecol. 2004 Jan;103(1):24-30.
14. Kozlowski CL, Lee D, Shwe KH, Love EM. Quantification of anti-c in haemolytic disease of the newborn. Transfus.Med. 1995 Mar;5(1):37-42.
15. Koelewijn JM, Vrijkotte TG, van der Schoot CE, Bonsel GJ, de HM. 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 May;48(5):941-52.
16. Roberts I.A.G. Haematological values in the newborn. In: Rennie J.M., editor. Roberton's textbook of neonatology. 4 ed. Philadelphia: Elsevier; 2011. p. 1287.
17. 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 Jan;89(1):57-60.

18. Dorn I, Schlenke P, Hartel C. Prolonged anemia in an intrauterine-transfused neonate with Rh-hemolytic disease: no evidence for anti-D-related suppression of erythropoiesis in vitro. Transfusion 2010 May;50(5):1064-70.
19. 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 Oct;199(4):387-4.
20. Filbey D, Hanson U, Wesstrom G. The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: a 12 year study in central Sweden. Acta Obstet.Gynecol.Scand. 1995 Oct;74(9):687-92.
21. Gottvall T, Filbey D. Alloimmunization in pregnancy during the years 1992-2005 in the central west region of Sweden. Acta Obstet.Gynecol.Scand. 2008;87(8):843-8.
22. Howard H, Martlew V, McFadyen I, Clarke C, Duguid J, Bromilow I, Eggington J. Consequences for fetus and neonate of maternal red cell allo-immunisation. Arch.Dis.Child Fetal Neonatal Ed 1998 Jan;78(1):F62-F66.
23. Karagol BS, Zenciroglu A, Okumus N, Karadag N, Dursun A, Hakan N. Hemolytic Disease of the Newborn Caused by Irregular Blood Subgroup (Kell, C, c, E, and e) Incompatibilities: Report of 106 Cases at a Tertiary-Care Centre. Am.J.Perinatol. 2012 Jun;29(6):449-54.
24. Nordvall M, Dziegiel M, Hegaard HK, Bidstrup M, Jonsbo F, Christensen B, Hedegaard M. Red blood cell antibodies in pregnancy and their clinical consequences: synergistic effects of multiple specificities. Transfusion 2009 Oct;49(10):2070-5.

Part 4

Associated hematological morbidity

Chapter 9

Thrombocytopenia at birth in neonates with red cell alloimmune hemolytic disease

Mirjam E.A. Rath & Vivianne E.H.J. Smits-Wintjens (contributed equally)
Dick Oepkes
Erik W. van Zwet
Inge L. van Kamp
Anneke Brand
Frans J. Walther
Enrico Lopriore

Vox Sang 2012; 102(3):228-233

Abstract

Objective

To evaluate the incidence and severity of and risk factors for thrombocytopenia at birth in neonates with red cell alloimmunization.

Study design

All neonates with hemolytic disease of the fetus/newborn (HDFN) due to red cell alloimmunization admitted to our center between January 2000 and September 2010 were included in this retrospective study. We measured platelet counts at birth and determined the incidence of thrombocytopenia (platelet count $<150 \times 10^9$/L) and severe thrombocytopenia (platelet count $<50 \times 10^9$/L). Risk factors for thrombocytopenia at birth were evaluated.

Results

Thrombocytopenia was present in 26% (94/362) of included neonates with HDFN at birth. Severe thrombocytopenia was found in 6% (20/362) of neonates. Three risk factors were found to be independently associated with thrombocytopenia at birth: treatment with intrauterine red cell transfusion (IUT) (OR 3.32, 95% CI 1.67-6.60, p = 0.001), small for gestational age (SGA) below the 10^{th} percentile (OR 3.32, 95% CI 1.25-8.80, p = 0.016), and lower gestational age at birth (OR 1.22 per week, 95% CI 1.02-1.44, p = 0.025).

Conclusions

Thrombocytopenia at birth occurs in 26% of neonates with HDFN due to red cell alloimmunization and is independently associated with IUT treatment, SGA and lower gestational age at birth.

Introduction

Limited studies have shown that fetuses with red cell alloimmunization are at increased risk of thrombocytopenia (platelet count <150 × 10^9/L).[1-3] In Rhesus D hemolytic disease treated with intrauterine red cell transfusion (IUT), thrombocytopenia was detected in 26% of fetuses at cordocentesis and was associated with fetal hydrops.[2] In Kell hemolytic disease, the incidence of fetal thrombocytopenia appears to be lower (10%) and less severe compared to fetuses with Rhesus D alloimmunization.[2,3]

Incidence and severity of thrombocytopenia in neonates with red cell alloimmunization at birth is unclear. In one small study (n = 20) thrombocytopenia was detected in 55% of neonates with Rhesus hemolytic disease during the neonatal period.[4] However, platelet count was not routinely measured at birth and possibly neonatal thrombocytopenia developed after birth due to treatment with exchange transfusion for hyperbilirubinemia.[5]

The exact cause of fetal and neonatal thrombocytopenia in red cell alloimmunization is not well known. Decreased production, increased destruction or a combination of both may play a role.[1,4,6] Common risk factors for fetal and neonatal thrombocytopenia such as preeclampsia, maternal diabetes and intrauterine growth retardation may also play a role in pregnancies affected by red cell alloimmunization.[7,8]

The aim of this study was to evaluate the incidence and severity of and risk factors for thrombocytopenia at birth in a large series of neonates with hemolytic disease of the fetus/newborn (HDFN) due to red cell alloimmunization.

Patients and Methods

All neonates with HDFN due to maternal red cell alloimmunization admitted between January 2000 and September 2010 at the Leiden University Medical Center (LUMC) were included in this retrospective observational study. Our center is the single national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. Part of the fetuses/neonates has been described in two previous studies on fetal thrombocytopenia,[2,3] two retrospective studies on transfusions in red cell alloimmunization[9,10] and in a randomized trial on the use of intravenous immunoglobulin.[11]

Our management guidelines in neonates with HDFN dictate that a full blood count (including hemoglobin level, reticulocyte count and platelet count) must be routinely performed in all neonates at birth. In addition, in the subgroup of fetuses treated with IUT, a full blood count is routinely performed at cordocentesis before each IUT to determine the desired amount of packed donor red cells.

Primary outcome was the incidence of and risk factors for thrombocytopenia at birth in neonates with HDFN.

Thrombocytopenia was defined as a platelet count <150 × 10^9/L and was classified as mild (101 to 149 × 10^9/L), moderate (51 to 100 × 10^9/L), severe (21 to 50 × 10^9/L) and very severe (≤20 × 10^9/L). A fully automated cell counter (Sysmex XE-2100), utilizing optical fluorescent platelet count in situations where an impedance count is unreliable, was used to determine fetal and neonatal platelet counts. A concentrated platelet transfusion (single donor plasma-reduced platelet apheresis concentrates) in a dose of 20 × 10^9/kg was given at birth if: (1) platelet count was <20 × 10^9/L (before November 2009 <30 × 10^9/L) in clinically stable neonates; (2) platelet count was <50 × 10^9/L in neonates with a manifest bleeding, those undergoing a procedure with risk of bleeding and in clinically unstable neonates with birth weight <1500 gram.

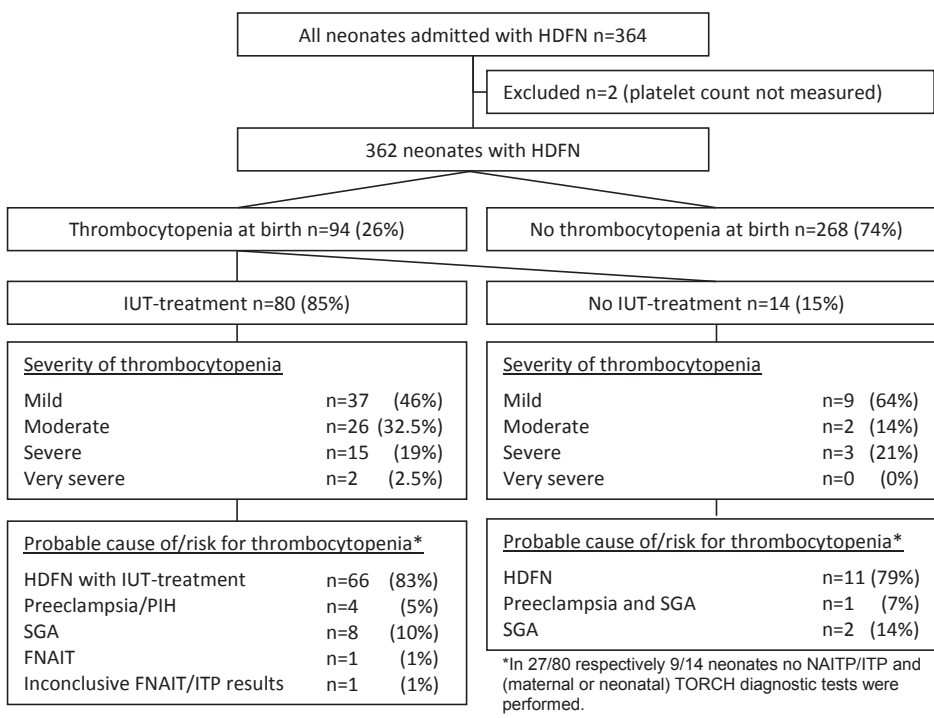
We recorded the following obstetric and neonatal data: type of red cell alloimmunization, number of IUTs, presence of fetal hydrops, fetal platelet count before each IUT, number of fetal platelet transfusions and neonatal platelet transfusions at birth, gestational age at birth, birth weight, small for gestational age (SGA) (defined as a birth weight <10^{th} percentile),[12] perinatal asphyxia (defined as Apgar score <7 at 5 minutes after birth), the presence of early onset neonatal sepsis (defined as clinical symptoms of infection and positive blood culture in the first 72 hours of life) and test results for TORCH infection and fetal/neonatal alloimmune thrombocytopenia (FNAIT). We recorded the presence of clinical signs of bleeding at birth and intracranial hemorrhage on the first cranial ultrasound performed within 24 hours after birth. A cranial ultrasound is performed on all IUT treated neonates. We documented the following maternal data: PIH (pregnancy induced hypertension)/preeclampsia, HELLP syndrome (syndrome of Hemolysis, Elevated Liver enzymes, Low Platelet counts) diabetes and TORCH infection.

Statistical analysis was performed using Student-t-test and Mann-Whitney test for continuous variables. Chi square and Fisher's exact tests were used for categorical variables, as appropriate. The following possible risk factors for thrombocytopenia at birth were included in a multivariate logistic regression model to measure independent effects: Rhesus D type of red cell alloimmunization, PIH/preeclampsia, HELLP syndrome, maternal diabetes, gestational age at birth, SGA, treatment with IUT, perinatal asphyxia and fetal hydrops. The results of the logistic model were expressed as odds ratios (OR). A p-value <0.05 was considered statistically significant. Statistical analysis was executed with SPSS 17.0 (SPSS Inc, Chicago, IL, USA).

Results

During the study period 364 neonates with HDFN of 330 mothers were admitted to our neonatal nursery. A flow chart of included neonates and information on severity and causes of thrombocytopenia at birth is presented in figure 1. A full blood count was measured in all but 2 neonates (99%, 362/364). In IUT treated neonates, a full blood count was measured in all but 2 fetuses (99%, 242/244) at cordocentesis. Baseline characteristics are summarized in table 1.

Figure 1. Flowchart showing numbers of neonates enrolled and severity and causes of thrombocytopenia at birth



Incidence, cause and severity of thrombocytopenia in HDFN

Incidence and severity of thrombocytopenia at birth

Thrombocytopenia was detected in 26% (94/362) of neonates at birth and was classified as mild (49%, 46/94), moderate (30%, 28/94), severe (19%, 18/94) and very severe (2%, 2/94). No neonates had clinical signs of bleeding at birth except for one hydropic premature

neonate (born at 30 weeks' gestation) with intraventricular hemorrhage grade 2 on day one. His platelet count at birth was 53 × 10^9/L.
Four percent (14/362) of neonates received a platelet transfusion at birth due to thrombocytopenia.

Table 1. Baseline characteristics of all included neonates with HDFN due to red cell alloimmunization

	Neonates with HDFN (n = 362)
Neonates treated with IUT, n (%)	244 (67)
Number of IUTs in IUT treated neonates[a]	3 (1-6)
Fetal thrombocytopenia before IUT, n (%)	42 (17)
Fetal thrombocytopenia*, n (%)	97 (40)
Gestational age at birth (weeks)[a]	36 (27-42)
Birth weight (grams)[b]	2904 ± 548
SGA (birth weight <p10), n (%)	22 (6)
Male, n (%)	228 (63)
Rhesus D alloimmunization, n (%)	268 (74)
Kell alloimmunization, n (%)	51 (14)
Rhesus c alloimmunization, n (%)	28 (8)
Rhesus E alloimmunization, n (%)	9 (3)
Fy(a) alloimmunization, n (%)	2 (1)
Cw alloimmunization, n (%)	2 (1)
Jk(a) alloimmunization, n (%)	1 (0)
Rhesus C alloimmunization, n (%)	1 (0)

[a] Value given as median (range); * Based on all fetal platelet counts before each IUT; [b] Value given as mean ± SD

Fetal thrombocytopenia and IUT in neonates with thrombocytopenia at birth

Eighty of the 94 thrombocytopenic neonates at birth received at least one IUT (figure 1). Thirty-one percent (24/78) and 95% (76/80) of IUT treated neonates with thrombocytopenia at birth had fetal thrombocytopenia based on the first fetal platelet count (before the first IUT) and all fetal platelet counts, respectively.
Only one (non-hydropic) fetus received an intrauterine platelet transfusion at a platelet count of 27 × 10^9/L in addition to a single IUT at 33 weeks' gestation. He was born after 35 4/7 weeks' gestation with a birth weight of 2580 gram (p25-p50) and Apgar scores of 4, 7 and 7 after 1, 5 and 10 minutes, respectively. Platelet count at birth was 22 × 10^9/L and he received one platelet transfusion on day one. Screening tests in this patient showed no evidence of FNAIT or TORCH congenital infection.

Diagnostic tests in neonates with thrombocytopenia at birth

Fifteen neonates with thrombocytopenia at birth were screened for fetal/neonatal alloimmune thrombocytopenia (FNAIT) and one neonate had FNAIT coinciding with her Rhesus D HDFN. Maternal and/or neonatal serologic screening tests for congenital TORCH infection were performed in 63% (59/94) of neonates with thrombocytopenia. All TORCH screening tests were negative. No cases of early onset neonatal sepsis were detected.

Risk factors for thrombocytopenia at birth

Detailed information on risk factors for thrombocytopenia at birth and blood results of neonates with and without thrombocytopenia at birth are summarized in table 2.

Univariate analysis:

Type of alloimmunization

The incidence of thrombocytopenia at birth in neonates with Rhesus D, Kell, Rhesus c and other types of red cell alloimmunization was 26% (69/268), 24% (12/51), 36% (10/28) and 20% (3/15), respectively. Type of red blood cell alloimmunization was not associated with thrombocytopenia at birth (table 2). The incidence of severe thrombocytopenia (platelet count $\leq 50 \times 10^9$/L) at birth was also not statistically different in neonates with Rhesus D, Kell, or Rhesus c compared to neonates without Rhesus D, Kell or Rhesus c respectively (p = 0.672, p = 0.434 and p = 0.696, respectively).

Perinatal risk factors

Several risk factors were associated with thrombocytopenia at birth including: treatment with IUT (OR 3.62, 95% CI 1.95-6.73, p = <0.001), fetal hydrops (OR 2.97, 95% CI 1.58-5.58, p = <0.001), PIH/preeclampsia (OR 7.36, 95% CI 1.40-38.6, p = 0.015), lower gestational age at birth (OR 1.33 for each week less, 95% CI 1.16-1.54, p = <0.001) and SGA (OR 3.10, 95% CI 1.30-7.40, p = 0.012).

Only one mother had HELLP syndrome and one mother had diabetes (both neonates had normal platelet counts at birth).

Multivariate analysis

On multivariate analysis, the following risk factors were independently associated with thrombocytopenia at birth: treatment with IUT, lower gestational age at birth and SGA (table 2). Fetal hydrops was not significant at the 5% level, but the relatively low p-value (p = 0.083) is suggestive of a possible independent association with thrombocytopenia at birth. Maternal diabetes and HELLP syndrome were excluded from multivariate analysis because

Table 2. Characteristics in neonates with HDFN with and without thrombocytopenia at birth

	Thrombocytopenia at birth (n = 94)	No Thrombocytopenia at birth (n = 268)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	p-value	OR (95% CI)	p-value
PIH/preeclampsia, n (%)	5 (5)	2 (1)[c]	7.36 (1.40-38.6)	0.015	4.48 (0.67-29.88)	0.122
Neonates treated with IUT, n (%)	80 (85)	164 (61)	3.62 (1.95-6.73)	<0.001	3.32 (1.67-6.60)	0.001
Number of IUTs in IUT treated neonates[a]	3 (2-4)	3 (2-4)		0.348		
Fetal thrombocytopenia at cordocentesis, n (%)	56/81 (69)	42/162 (26)[d]	6.51 (3.62-11.71)	<0.001		
mild to moderate, n (%)	49/56 (88)	40/42 (95)				
severe to very severe, n (%)	7/56 (13)	2/42 (5)				
Fetal hydrops, n (%)	22 (23)	25 (9)	2.97 (1.58-5.58)	<0.001	1.88 (0.92-3.83)	0.083
Gestational age at birth, weeks[a]	36 (35-37)	37 (36-37)	1.33 (1.16-1.54) for each week less	<0.001	1.22 (1.02-1.44) for each week less	0.025
Birth weight, grams[b]	2672 ± 617	2986 ± 497	0.35 (0.22-0.55)	<0.001		
SGA (birth weight <p10), n (%)	11 (12)	11 (4)	3.10 (1.30-7.40)	0.012	3.32 (1.25-8.80)	0.016
Apgar score at 5 min <7, n (%)	4 (5)[e]	4 (2)[f]	2.96 (0.72-12.06)	0.212	1.13 (0.23-5.42)	0.882
Male, n (%)	66 (70)	162 (60)	1.54 (0.93-2.56)	0.092		
Rhesus D alloimmunization, n (%)	69 (73)	199 (74)	0.96 (0.56-1.63)	0.872	0.81 (0.44-1.48)	0.489
Kell alloimmunization, n (%)	12 (13)	39 (15)	0.86 (0.43-1.72)	0.668		
Rhesus c alloimmunization, n (%)	10 (11)	18 (7)	1.65 (0.73-3.72)	0.221		
Other type of red cell alloimmunization, n (%)	3 (3)	12 (5)		0.768		
Hemoglobin level at birth, g/dL[b]	11.4 ± 3.2	12.4 ± 2.8	0.81 (0.71-0.94)	0.003		
Reticulocyte count at birth, ‰[a]	7.5 (2-76.25)[g]	43 (5.5-78.5)[h]		0.835		
Platelet count at birth, 10^9/L[b]	93.3 ± 40.2	254.4 ± 68.0		<0.001		

[a] Value given as median (IQR), [b] Value given as mean ± SD, [c] Assessed in 264/268 neonates, [d] Assessed in 162/164 neonates, [e] Assessed in 93/94, [f] Assessed in 267/268 neonates, [g] Assessed in 50/94 neonates, [h] Assessed in 165/268 neonates

of limited number of cases. As birth weight and gestational age at birth are closely related, birth weight was also excluded from multivariate analysis.

Discussion

This study demonstrates that thrombocytopenia at birth is common among neonates with HDFN due to red cell alloimmunization, occurring in 26% of neonates compared to 1-5% in the general population and 22-35% in the neonatal intensive care unit (NICU) population.[13] Severe thrombocytopenia was present in 6% of all neonates with HDFN compared to 5-10% in the NICU population.

This is the first study describing the incidence of neonatal thrombocytopenia at birth in HDFN due to red cell alloimmunization. Koenig et al. described neonatal thrombocytopenia in 11 of 20 (55%) neonates with Rhesus HDFN during admission.[4] In this study, platelet count was not routinely measured at birth and in several cases only after exchange transfusion. Exchange transfusion is a known risk factor for thrombocytopenia, independently of red cell alloimmunization.[5,14]

We found a positive association between IUT treatment and thrombocytopenia at birth. The cause of this association is not clear and several mechanisms may play a role. Increased erythropoiesis could theoretically lead to suppression of thrombopoiesis by the hematopoietic stem cells.[1,4] However, since IUT is known to suppress erythropoiesis,[15] this theory only supports fetal thrombocytopenia at the first IUT. Increased incidence of fetal thrombocytopenia from 17% to 40% in fetuses treated with several IUTs may be explained by a decreased production, increased consumption, increased destruction, or dilution. In addition, IUT with packed red cells can cause dilution of platelets.[16] However, it is unlikely that this effect is still present at the time of a consecutive IUT after two to three weeks.

We found that type of red cell alloimmunization was not a risk factor for thrombocytopenia at birth. In a previous study fetal thrombocytopenia (at first IUT) appeared to be less common in Kell HDFN than in Rhesus D HDFN.[3] The discrepancy between the results may be due to several factors including methodological differences between the two studies. The higher rate of thrombocytopenia in Kell HDFN found in this study may be due to the higher number of IUTs in the Kell population.[10]

Prematurity and intrauterine growth restriction have previously been described as risk factors for early-onset (<72 hours) neonatal thrombocytopenia.[7,8] In accordance, we demonstrated that lower gestational age at birth and SGA are independent risk factors for thrombocytopenia at birth in neonates with HDFN. In addition, we found that lower birth weight irrespective of gestational age is a risk factor for thrombocytopenia at birth in red cell alloimmunization.

Perinatal asphyxia (Apgar score <7), maternal PIH/preeclampsia and syndrome of HELLP have formerly been described as risk factors for thrombocytopenia at birth.[7,17,18] In our study population perinatal asphyxia was not associated with thrombocytopenia at birth and the number of cases with PIH/preeclampsia was limited.

Interestingly, one case of thrombocytopenia in this cohort was found to be due to FNAIT. Four other case reports of thrombocytopenia due to FNAIT have been described in fetuses/neonates with Rhesus hemolytic disease.[19-22]

Fortunately only one neonate had clinical signs of bleeding at birth (intraventricular hemorrhage grade 2). Although this neonate was thrombocytopenic at birth, in this case other factors such as prematurity and hydrops could have contributed to this bleeding complication. Moreover, the causal relation between thrombocytopenia and intraventricular hemorrhage is controversial.[23]

The results of this study should be interpreted with care because of the retrospective study design. We have not systematically investigated all other possible causes of neonatal thrombocytopenia such as maternal immune thrombocytopenic purpura (ITP), FNAIT and perinatal/neonatal infection. Hence the incidence of 26% of thrombocytopenia at birth due to red cell alloimmunization can be an overestimate. Finally, the number of spurious thrombocytopenia because of clotted samples and platelet clumping is unclear.

In conclusion, this study shows that 26% of neonates with HDFN due to red cell alloimmunization have thrombocytopenia at birth. Risk for thrombocytopenia is independently associated with IUT treatment, SGA and lower gestational age at birth.

References

1. Saade GR, Moise KJ, Jr., Copel JA, et al.: Fetal platelet counts correlate with the severity of the anemia in red-cell alloimmunization. Obstet Gynecol 1993; 82(6):987-991.
2. Van den Akker ES, de Haan TR, Lopriore E, et al.: Severe fetal thrombocytopenia in Rhesus D alloimmunized pregnancies. Am J Obstet Gynecol 2008; 199(4):387-4.
3. Van den Akker ES, Klumper FJ, Brand A, et al.: Kell alloimmunization in pregnancy: associated with fetal thrombocytopenia? Vox Sang 2008; 95(1):66-69.
4. Koenig JM, Christensen RD: Neutropenia and thrombocytopenia in infants with Rh hemolytic disease. J Pediatr 1989; 114(4 Pt 1):625-631.
5. Chadd MA, Gray OP, Hole DJ: Blood coagulation studies during exchange transfusion. J Obstet Gynaecol Br Commonw 1972; 79(4):373-376.
6. Wagner T, Bernaschek G, Geissler K: Inhibition of megakaryopoiesis by Kell-related antibodies. N Engl J Med 2000; 343(1):72.
7. Murray NA, Roberts IA: Circulating megakaryocytes and their progenitors in early thrombocytopenia in preterm neonates. Pediatr Res 1996; 40(1):112-119.
8. Watts T, Roberts I: Haematological abnormalities in the growth-restricted infant. Semin Neonatol 1999; 4(1):41-54.
9. Rath ME, Smits-Wintjens VE, Lindenburg I, et al.: Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. Vox Sang 2010; 99(1):65-70.
10. Rath ME, Smits-Wintjens VE, Lindenburg IT, et al.: Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2010; 100(3):312-6.
11. Smits-Wintjens VE, Walther FJ, Rath ME, et al.: Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011; 127(4):680-686.
12. Kloosterman GJ: [Intrauterine growth and intrauterine growth curves]. Ned Tijdschr Verloskd Gynaecol 1969; 69(5):349-365.
13. Roberts I, Stanworth S, Murray NA: Thrombocytopenia in the neonate. Blood Rev 2008; 22(4):173-186.
14. 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(4):265-271.
15. De Boer IP, Zeestraten EC, Lopriore E, et al.: Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. Am J Obstet Gynecol 2008; 198(1):54.
16. Viëtor HE, Klumper F, Meerman RJ, et al.: Intrauterine transfusions influence fetal leukocyte counts and subsets. Prenat Diagn 1998; 18(4):325-331.
17. Beiner ME, Simchen MJ, Sivan E, et al.: Risk factors for neonatal thrombocytopenia in preterm infants. Am J Perinatol 2003; 20(1):49-54.
18. Harms K, Rath W, Herting E, et al.: Maternal hemolysis, elevated liver enzymes, low platelet count, and neonatal outcome. Am J Perinatol 1995; 12(1):1-6.
19. Carbonne B, Chereau E, Larsen M, et al.: Concomitant fetal anemia and thrombocytopenia due to anti-D and anti-HPA1a alloimmunization. Prenat Diagn 2005; 25(12):1172-1174.
20. Klüter H, Germer U, Gortner L, et al.: Coincidence of neonatal alloimmune thrombocytopenia and maternal anti-D immunization: case report. Br J Haematol 1998; 102(5):1383-1384.

21. Schild RL, Hoch J, Plath H, et al.: Perinatal management of fetal hemolytic disease due to Rh incompatibility combined with fetal alloimmune thrombocytopenia due to HPA-5b incompatibility. Ultrasound Obstet Gynecol 1999; 14(1):64-67.
22. Yeast JD, Plapp F: Fetal anemia as a response to prophylactic platelet transfusion in the management of alloimmune thrombocytopenia. Am J Obstet Gynecol 2003; 189(3):874-876
23. Baer VL, Lambert DK, Henry E, et al.: Severe Thrombocytopenia in the NICU. Pediatrics 2009; 124(6):e1095-e1100.

Chapter 10

Cholestasis in neonates with red cell alloimmune hemolytic disease: incidence, risk factors and outcome

Vivianne E.H.J. Smits-Wintjens
Mirjam E.A. Rath
Irene T.M. Lindenburg
Dick Oepkes
Erik W. van Zwet
Frans J. Walther
Enrico Lopriore

Neonatology 2012; 101(4):306-310

Abstract

Background

Etiology of cholestatic liver disease in neonates with hemolytic disease of the newborn (HDN) has been associated with iron overload due to intrauterine red cell transfusions (IUTs). Data on the incidence and severity of cholestasis in neonates with HDN is scarce, and little is known about pathogenesis, risk factors, neonatal management and outcome.

Objective

To evaluate incidence, risk factors, management and outcome of cholestasis in neonates with red cell alloimmune hemolytic disease.

Methods

All (near-) term neonates with HDN due to red cell alloimmunization admitted to our center between January 2000 and July 2010 were included in this observational study. Liver function tests (including conjugated bilirubin) were routinely performed in the neonatal period. We recorded the presence of cholestasis, investigated several potential risk factors and evaluated the management and outcome in affected neonates.

Results

A total of 313 infants with red cell alloimmune hemolytic disease treated with or without IUTs were included. The incidence of cholestasis was 13% (41/313). Two risk factors were independently associated with cholestasis: treatment with at least one IUT (OR 5.81, 95% CI 1.70-19.80, p = 0.005) and Rhesus D type of alloimmunization (OR 4.66, 95% CI 1.05-20.57, p = 0.042). Additional diagnostic tests to investigate possible causes of cholestasis were all negative. In five infants (12%) supportive medical and nutritional therapy was started and one neonate required iron chelation therapy.

Conclusion

Cholestasis occurs in 13% of neonates with HDN due to red cell alloimmunization and is independently associated with IUT treatment and Rhesus D type of alloimmunization.

Introduction

Hemolytic disease of the newborn (HDN) due to red cell alloimmunization may lead to excessive unconjugated hyperbilirubinemia, anemia and iron overload.[1] A few studies have reported an association between HDN and the development of conjugated hyperbilirubinemia, i.e. cholestasis.[2-5] Some of these studies (mostly case reports) describe that cholestasis in neonates with HDN is uncommon and usually mild and transient.[3-5] Other reports however detail severe and protracted courses of cholestasis.[2,4,6,7] The etiology of cholestatic liver disease in neonates with HDN has been associated with iron overload due to intrauterine transfusions (IUTs).[6,8-10] Data on the incidence and severity of cholestasis in neonates with red cell alloimmune hemolytic disease is scarce, and little is known about pathogenesis, risk factors, neonatal management and outcome.
The aim of this study was to evaluate incidence, potential risk factors, management and outcome of cholestasis in a large series of neonates with HDN due to red cell alloimmunization.

Patients and Methods

All consecutive cases of (near-) term neonates (≥35 weeks of gestation) with HDN due to maternal red cell alloimmunization admitted to our center between January 2000 and July 2010 were included in this retrospective study. Neonatal outcome in part of this group was described in previous studies.[11-14] The Leiden University Medical Center (LUMC) is the national referral center for the management and intrauterine treatment of red cell alloimmunization in the Netherlands. We excluded all preterm neonates (<35 weeks of gestation) and neonates in whom conjugated bilirubin tests were not performed. The guidelines for the management of neonates with HDN admitted to our nursery (including intensive phototherapy and exchange transfusion (ET)) have previously been described.[12,13] In addition to frequent total bilirubin levels, extensive diagnostic evaluations are routinely performed at birth and during the first week of life in infants with red cell alloimmune HDN admitted to our neonatal nursery. These evaluations include hematologic tests (complete blood counts), liver function tests (liver enzymes and total and conjugated bilirubin) and blood group, Coombs and irregular antibody tests.
Primary outcome of this study was the incidence of cholestatic icterus in neonates with HDN due to red cell alloimmunization. Secondary outcomes were management and outcome of cholestasis.

Cholestasis or conjugated hyperbilirubinemia was defined as a conjugated serum bilirubin level above 1.0 mg/dL if total serum bilirubin level is less than 5 mg/dL, or a value of conjugated bilirubin that represents more than 20% of total bilirubin if the total bilirubin level is greater than 5 mg/dL.[15] Severe cholestasis was defined as a conjugated bilirubin level >50% of the total serum bilirubin concentration.

In neonates with cholestasis we recorded the following data: symptoms of cholestasis (such as discolored stools and dark urine), duration of conjugated hyperbilirubinemia, (type of) therapy (including Ursodeoxycholic acid (15 mg/kg/d), Vitamin A (2500-5000 IU/d, in prematures 1000-1500 IU/kg/d), vitamin D (800 IU/d, in prematures 400 IU/d), vitamin E (5-10 mg/kg/d, in prematures 10-20 mg/kg/d), vitamin K (1 mg/d, birth weight <1500 grams: 0.5 mg/d) and formula with medium chain triglycerides) and investigations performed to establish a specific cause for the cholestatic icterus. Possible causes for neonatal cholestasis are (1) infections (sepsis, urinary tract infection, toxoplasmosis, rubella, cytomegalovirus, human herpes virus 6, syphilis, parvovirus B19, echovirus, adenovirus, coxsackie virus, hepatitis B and C); (2) bile duct anomalies, including biliary atresia and choledochal cyst; (3) inborn errors of metabolism, including alpha-1-antitrypsin deficiency, galactosemia, cystic fibrosis, tyrosinemia and progressive familial intrahepatic cholestasis, and (4) endocrinopathies (hypothyroidism and hypopituitarism).

We recorded the following obstetric and neonatal data: type of red cell alloimmunization, number of IUTs, gestational age at birth, birth weight, total bilirubin level and conjugated bilirubin level at birth, maximum total bilirubin level and maximum conjugated bilirubin level during admission, time until cholestasis disappeared (within 1 week, between 1 week and 1 month or after 1 month), maximum ferritin level during admission, duration of phototherapy, number of ETs required and number of top up red blood cell transfusions received during the first 3 months of life.

Data are reported as means and standard deviations (SD) or as medians and ranges. Statistical analysis was performed using Student-t test and Mann-Whitney test for continuous variables. Chi-square and Fisher's-exact test were used for categorical variables. To assess the relationship between ferritin level and treatment with IUT a Spearman correlation was calculated. A p-value <0.05 was considered to indicate statistical significance. All predicting risk factors for cholestasis identified with univariate analysis were included in a multivariate logistic regression model to measure independent effects. The results of the logistic models were expressed as odds ratios (OR). Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

During the study period 357 neonates with HDN due to red cell alloimmunization were admitted to our neonatal nursery. We excluded 35/357 (10%) neonates due to prematurity (<35 weeks of gestation) and 9/357 (3%) neonates because conjugated bilirubin levels were not available. A total of 313 patients were included in this study. Forty-one neonates (13%) met the criteria for cholestasis (cholestasis group). Baseline characteristics are summarized in Table 1.

Table 1. Baseline characteristics

	Neonates with HDN (n = 313)
Neonates treated with IUT, n (%)	206 (66)
Number of IUTs in IUT treated neonates[a]	3 (1-6)
Gestational age at birth, weeks[a]	37 (35-42)
Birth weight, kg[b]	3.0 ± 0.4
Male, n (%)	197 (63)
Type of red cell alloimmunization	
Rhesus D, n (%)	240 (76.7)
Rhesus C, n (%)	2 (0.6)
Rhesus c, n (%)	24 (7.7)
Rhesus E, n (%)	7 (2.2)
Kell, n (%)	38 (12.1)
Cw, n (%)	1 (0.3)
Jk(a), n (%)	1 (0.3)

[a] Value given as median (range), [b] Value given as mean ± SD

Risk factors for cholestasis

Detailed information on risk factors for cholestasis is summarized in Table 2.[16,17] Eighty-eight percent of neonates with cholestasis had both Rhesus D type of alloimmunization and were treated with IUT.

Univariate analysis

Several risk factors were found to be associated with cholestasis, including: lower birth weight (OR 3.70 for each kg less, 95% CI 1.61-8.33, p = 0.001 and OR 1.92 for each 500 grams less, 95% CI 1.27-2.89, p = 0.002), Rhesus D type of alloimmunization (OR 6.89, 95% CI 1.62-29.26, p = 0.009), treatment with IUT (OR 7.84, 95% CI 2.36-26.05, p = <0.001),

Table 2. Analysis of potential risk factors for cholestasis in neonates with HDN

	Non-cholestasis group (n = 272)	Cholestasis group (n = 41)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	p-value	OR (95% CI)	p-value
Gestational age at birth, weeks[a]	37 (35-42)	36 (35-38)	1.30 (0.90-1.89) for each week less	0.230		
Birth weight, kg[b]	3.0 ± 0.4	2.8 ± 0.4	3.70 (1.61-8.33) for each kg less	0.001	2.34 (0.95-5.78) for each kg less	0.066
Rhesus D alloimmunization, n (%)	201 (73.8)	39 (95.1)	6.89 (1.62-29.26)	0.003	4.66 (1.05-20.57)	0.042
Rhesus c alloimmunization, n (%)	23 (8.5)	1 (2.4)	0.27 (0.04-2.06)	0.339		
Kell alloimmunization, n (%)	37 (13.6)	1 (2.4)	0.16 (0.02-1.19)	0.040		
Neonates treated with IUT, n (%)	168 (62)	38 (93)	7.84 (2.36-26.05)	<0.001	5.81 (1.70-19.80)	0.005
Number of IUTs in IUT treated neonates[a]	3 (1-6)	3 (1-6)		0.105		
Hemoglobin level at birth, g/dL[b,c]	12.3 ± 2.9	11.2 ± 2.6	1.17 (1.03-1.33) for each g/dL less	0.016		
Bilirubin level at birth, mg/dL[b,d]	5.6 ± 2.5	7.3 ± 4.3	1.19 (1.07-1.33)	0.004		
Maximum bilirubin level, mg/dL[b]	13.2 ± 4.7	14.8 ± 6.3		0.085		
Conjugated bilirubin level at birth, mg/dL[b,e]	0.6 ± 0.3	2.9 ± 3.0		<0.001		
Maximum conjugated bilirubin level, mg/dL[b]	1.0 ± 0.6	7.1 ± 6.7		<0.001		
Phototherapy, days[b]	4.2 ± 2.0	4.1 ± 1.8		0.802		
Maximum ferritin level, µg/L[a,f]	657 (86-10195)	1191 (489-73000)	1.04 (0.99-1.08) per 100 µg/L more	<0.001		
Neonates treated with exchange transfusion, n (%)	106 (39)	21 (51)		0.170		
Number of exchange transfusions per neonate[a]	0 (0-5)	1 (0-2)		0.144		
Neonates treated with top up transfusion, n (%)	196 (72)	32 (78)		0.440		
Number of top up transfusions per neonate[a]	1 (0-6)	2 (0-6)	1.43 (1.15-1.77)	0.004	1.24 (0.98-1.57)	0.069

[a] Value given as median (range), [b] Value given as mean ± SD, [c] Reference range 34-40 weeks of gestation: 15.0-16.8 g/dL[17], [d] Reference range: <5.8 mg/dL[16], [e] Reference range: <0.23 mg/dL[16], [f] Reference range: 36-483 µg/L[16]

total serum bilirubin level at birth (OR 1.19, 95% CI 1.07-1.33, p = 0.004), maximum ferritin level (OR 1.04 per 100 µg/L more, 95% CI 0.99-1.08, p = <0.001) and number of top up transfusions (OR 1.43, 95% CI 1.15-1.77, p = 0.004).

Multivariate analysis

On multivariate analysis, the following risk factors were independently associated with cholestasis: Rhesus D type of alloimmunization (OR 4.66, 95% CI 1.05-20.57, p = 0.042) and treatment with IUT (OR 5.81, 95% CI 1.70-19.80, p = 0.005).

Because a higher total bilirubin level at birth is part of the definition of cholestasis and thus closely related to a higher conjugated bilirubin level, total bilirubin level at birth was excluded from multivariate analysis. As ferritin levels were determined only in 89/313 (28%) neonates and ferritin level and treatment with IUT were positively correlated (r = 0.565, p = <0.001), ferritin was not included in the multivariate analysis.

Clinical characteristics and outcome of cholestasis

In the cholestasis group 11/41 infants (27%) had severe cholestasis with a conjugated bilirubin level >50% of the total serum bilirubin concentration. Four neonates (10%) had symptoms of cholestasis such as discolored stools or dark urine. In 15% (6/41) the cholestasis disappeared spontaneously within 1 week, in 15% (6/41) between 1 week and 1 month and in 15% (6/41) within 1 to 3 months. In the remaining 56% (23/41) of infants the time of disappearance of cholestasis is not clear due to incomplete follow up. However, only 9% (2/23) of neonates with incomplete follow up had severe cholestasis. In five infants (12%) supportive medical and nutritional therapy was started (Ursodeoxycholic acid, Vitamin A, D, E, K and/or formula with medium chain triglycerides). In one infant Ursodeoxycholic acid was given for a period of 18 days, in the remaining 4 infants duration of therapy is not known since they were transferred to other hospitals while they were still on medication.

One patient with Rhesus D alloimmunization, who received 6 IUTs, developed severe cholestasis (maximum bilirubin level 41.3 mg/dL and maximum conjugated bilirubin level 35.1 mg/dL) and severe hyperferritinemia (maximum serum ferritin level 73000 µg/L). Iron chelation therapy with desferrioxamine was started and continued for one month to reduce the serum ferritin concentration and liver iron contents. After having excluded other causes of cholestasis, the most probable explanation for the cholestasis in this case was hyperferritinemia with iron overload in the liver, due to multiple IUTs.

Additional investigations in cholestasis group

In the cholestasis group, laboratory investigations to evaluate possible liver injury were performed in 36/41 infants (88%). Elevated levels for alkaline phosphatase were detected in 6 (15%), for aspartate aminotransferase (AST) in 17 (41%), for alanine aminotransferase (ALT) in 13 (32%) and for gamma-glutamyl transpeptidase (γGT) in 8 infants (20%).[16]
In 18/41 (44%) neonates in the cholestasis group additional tests were performed to investigate possible causes of cholestasis. Sixteen infants (39%) were screened for infection. In all of them bacterial cultures of blood and urine were negative and there were no proven infections with toxoplasmosis, rubella, cytomegalovirus, human herpes virus 6, syphilis, parvovirus B19, echovirus, adenovirus, coxsackie virus and hepatitis B and C. Additional tests to exclude endocrinologic or metabolic disorders were performed in 9/41 (22%) and 7/41 (17%) of infants, respectively. In none of these infants an endocrinopathy and/or an inborn error of metabolism was diagnosed. In 12/41 (29%) neonates an abdominal ultrasound was performed to exclude impairments in bile flow. All infants had normal ultrasound findings.

Discussion

This study shows that cholestasis is a common problem in HDN, occurring in 13% of neonates. Cholestasis is found particularly in neonates with Rhesus D alloimmunization treated with IUTs. Although cholestasis was mild and transient in most cases, a few neonates had severe cholestatic liver disease with protracted course and required intensive treatment and in one case chelation therapy was needed.
In the past, several studies have been published on the co-occurrence of cholestasis in neonates with HDN due to red cell alloimmunization. In 1963, Dunn described a large case series of 133 infants with Rhesus HDN and found that 8% of these patients developed 'obstructive jaundice' defined as conjugated bilirubin level >3 mg/dL.[3,4] However, in addition to the more stringent definition, their study is not fully comparable with contemporary care strategies for Rhesus HDN. In 1963 perinatal mortality and morbidity were far higher than nowadays, due to the absence of RhD prophylaxis, Doppler ultrasound to detect fetal anemia and in particular treatment with IUTs. Later, Bowman et al., Perez et al. and Allgood et al. also published on cholestasis in neonatal HDN, but none of these studies reported an exact incidence of cholestasis.[2,5,18]
We found that treatment with IUT is an independent risk factor for cholestasis. This could be due to iron overload which have been reported in neonates with HDN who underwent IUT.[6,8-10] In 1990 Berger and colleagues demonstrated elevated ferritin levels in 12 infants

with Rhesus HDN and suggested that iron overload could be an explanation for cholestatic icterus in Rhesus HDN.[9] In 1991, Nasrat et al. measured higher fetal plasma ferritin concentrations in 23 Rhesus alloimmunized fetuses compared to controls and serial IUTs were associated with additional increases in serum ferritin.[10] On the contrary, in 2004 Aygun et al. found higher cord blood ferritin levels in neonates affected with Rhesus HDN compared to birth weight and gestational age matched controls, but IUTs did not affect the ferritin status of the babies with Rhesus HDN.[8] This finding is in contrast with our observations. We found a positive correlation between treatment with one or more IUTs and high ferritin levels during admission, both risk factors for cholestasis in this study. Our data support the hypothesis of iron overload as a mechanism of cholestasis in HDN.

In addition to iron overload, the following etiologic mechanisms of cholestasis in HDN were previously described: overload of pigment causing stasis and blocking of bile canaliculi; liver necrosis caused by hypoxia due to anemia; and pressure by extramedullary hematopoiesis in the liver caused by anemia leading to damage of intrahepatic canaliculi.[19] Hence, the finding that IUT treatment is a risk factor for cholestasis could be due to the disease severity (more severe anemia necessitating IUT), to transfusion induced iron overload or to a combination of both. Theoretically, other causes such as infection or metabolic diseases or total parenteral nutrition may play a role. However, extensive investigations to rule out other causes of cholestasis in infants with cholestasis included in this study yielded no additional information.

This study shows that Rhesus D type of alloimmunization is an independent risk factor for cholestasis. This finding has not been described before. However, the vast majority (88%) of neonates within the cholestasis group had both Rhesus D type of alloimmunization and was treated with IUT, preventing reliable distinction between the actual role of both risk factors.

In our series cholestasis resolved spontaneously within 1 week to 3 months after birth in almost half of the patients, which is comparable with other studies.[2,18] In 56% of the included infants, conjugated bilirubin levels and liver enzyme levels (AST, ALT, γGT, and alkaline phosphatase) were not monitored until they reached normal values. We recommend to measure conjugated bilirubin levels and liver enzyme levels during the first three months of life or until they reach normal values.

We suggest that a full work-up to exclude other causes of cholestasis in a child with red cell alloimmnune HDN treated with at least one IUT, is not necessary, provided that no other factors are involved and monitoring of ferritin, liver enzymes and conjugated bilirubin levels is guaranteed during the first 3 months of life.

The results of this study should be interpreted with care due to the relatively small number of neonates in the cholestasis group and the retrospective study design. In addition,

our conclusions are limited due to incomplete measurements. For example, only 17% of neonates with cholestasis were tested for metabolic conditions and some of them may have had alpha-1-antitrypsin deficiency. Larger, multicenter studies are required to confirm our findings.

In conclusion, we found a 13% incidence of cholestasis in HDN due to red cell alloimmunization and identified several risk factors for cholestasis, in particular treatment with IUT and Rhesus D type of alloimmunization. Larger follow-up studies are required to determine the exact course and etiology of cholestasis in infants with red cell alloimmune hemolytic disease.

References

1. 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.
2. Allgood C, Bolisetty S: Severe conjugated hyperbilirubinaemia and neonatal haemolysis. Int J Clin Pract 2006;60:1513-1514.
3. Dunn PM: Rh Haemolytic Disease of the Newborn, 1960-1961. Arch Dis Child 1963;38:596-599.
4. Dunn PM: Obstructive Jaundice and Haemolytic Disease of the Newborn. Arch Dis Child 1963;38:54-61.
5. Perez EM, Cooper TR, Moise AA, Ferry GD, Weisman LE: Treatment of obstructive jaundice in erythroblastosis fetalis with ursodeoxycholic acid (UDCA): a case report. J Perinatol 1998;18:317-319.
6. Lasker MR, Eddleman K, Toor AH: Neonatal hepatitis and excessive hepatic iron deposition following intrauterine blood transfusion. Am J Perinatol 1995;12:14-17.
7. Yilmaz S, Duman N, Ozer E, Kavas N, Oren H, Demircioglu F, Kumral A, Ozkan H, Irken G, Ozer E: A case of rhesus hemolytic disease with hemophagocytosis and severe iron overload due to multiple transfusions. J Pediatr Hematol Oncol 2006;28:290-292.
8. Aygun C, Tekinalp G, Gurgey A: Increased fetal iron load in rhesus hemolytic disease. Pediatr Hematol Oncol 2004;21:329-333.
9. 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.
10. Nasrat HA, Nicolini U, Nicolaidis P, Letsky EA, Gau G, Rodeck CH: The effect of intrauterine intravascular blood transfusion on iron metabolism in fetuses with Rh alloimmunization. Obstet Gynecol 1991;77:558-562.
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;198:54.e1-54.e4.
12. Rath ME, Smits-Wintjens VE, 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.
13. Rath ME, Smits-Wintjens VE, Lindenburg IT, Brand A, Van Kamp IL, Oepkes D, Walther FJ, Lopriore E: Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang 2011;100:312-316.
14. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D, Brand A, Lopriore E: Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011;127:680-686.
15. Moyer V, Freese DK, Whitington PF, Olson AD, Brewer F, Colletti RB, Heyman MB: Guideline for the evaluation of cholestatic jaundice in infants: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2004;39:115-128.
16. Ayling RM, Carragher F: Neonatal biochemical reference ranges (appendix 6) in: Rennie JM, editor. Roberton's Textbook of Neonatology, ed 4. Philadelphia, Elsevier, 2005:1299-1308.
17. Roberts IAG: Haematological values in the newborn (appendix 1) in: Rennie JM, editor. Roberton's Textbook of Neonatology, ed 4. Philadelphia, Elsevier, 2005:1287.
18. Bowman JM: Another cause of neonatal cholestasis. J Pediatr 1986;108:489.
19. Sivan Y, Merlob P, Nutman J, Reisner SH: Direct hyperbilirubinemia complicating ABO hemolytic disease of the newborn. Clin Pediatr (Phila) 1983;22:537-538.

Chapter 11

Iron status in infants with alloimmune hemolytic disease in the first three months of life

Mirjam E.A. Rath
Vivianne E.H.J. Smits-Wintjens
Dick Oepkes
Frans J. Walther
Enrico Lopriore

Submitted

Abstract

Background and Objectives

Ferritin levels are often highly elevated at birth in neonates with alloimmune hemolytic disease of the fetus and newborn (HDFN). Data on ferritin levels in these infants in the first three months of life are lacking. Objective of this study was to examine the course of iron status and incidence of iron deficiency and overload in neonates with alloimmune HDFN up to three months of age. Secondary objective was to analyze bilirubin levels, liver enzymes and red blood cell indices in the same time period and the association with intrauterine transfusion (IUT).

Materials and Methods

Observational study of neonates with alloimmune HDFN admitted to our center between November 2010 and March 2012. Data on iron status, bilirubin levels, liver enzymes and red blood cell indices up to three months of age were collected prospectively and compared between neonates treated with and without IUT.

Results

Thirty-five infants with alloimmune HDFN were included. Iron overload occurred in 70% of neonates at birth and in 50% and 18% at the age of one and three months, respectively. No cases of iron deficiency at birth and only one case of iron deficiency at three months of age were found. No infants received iron therapy. Infants who received IUT had a significantly lower hemoglobin level and reticulocyte count and higher ferritin level at birth.

Conclusion

The vast majority of neonates with alloimmune HDFN have iron overload at birth. Incidence of iron overload gradually decreases within the first three months without iron supplementation.

Introduction

Iron supplementation therapy is occasionally given to support erythropoiesis in neonates with hemolytic disease of the fetus and newborn (HDFN) due to red cell alloimmunization.[1] However, iron stores in neonates with alloimmune HDFN are often not depleted at birth. On the contrary, ferritin levels in fetal and cord blood are often highly elevated.[2-4] Increased fetal and neonatal iron stores are most probably caused by the increased rate of fetal hemolysis.[4,5] Moreover, intrauterine red blood cell transfusions (IUTs) and/or postnatal red blood cell transfusions (top-up transfusions) can further increase iron overload.[6,7] Iron overload can cause damage to the liver, heart and endocrine organs, alter immune response and increase susceptibility to infection.[8] Nevertheless, iron is also crucial for early brain development and function. Iron deficiency as well as iron overload have been associated with neurodevelopmental impairment.[8]

Iron overload is most accurately diagnosed by measuring tissue iron using liver (and cardiac) biopsies.[9] However, these procedures are invasive, expensive and difficult to perform in neonates. Magnetic resonance imaging (MRI) and magnetic susceptometry are noninvasive methods to measure tissue iron stores, but are also expensive and complex.[10] Serum ferritin, which reflects total body iron capacity, is the most convenient and cost-effective technique to diagnose iron overload. An important limitation of serum ferritin levels as the sole marker for iron overload is that they can be influenced by inflammation, infection, liver damage, hemolysis, ineffective erythropoiesis and ascorbate deficiency independently of changes in body iron.[9,10]

Although it is known that ferritin levels at birth are frequently elevated, the course of iron status after birth in infants with HDFN is not clear. Primary aim of this study was to examine the course of iron status in infants with alloimmune HDFN. Secondary aim was to measure the course of bilirubin levels, liver enzymes, red blood cell indices, the incidence of iron overload and cholestatic disease and the association with IUT.

Materials and Methods

All term and preterm neonates with HDFN due to maternal red cell alloimmunization admitted to our center between November 2010 and March 2012 were eligible for this prospective observational study. The Leiden University Medical Center (LUMC) is the national referral center for the management and intrauterine treatment of HDFN. Informed consent was obtained to collect data from outpatient clinics. We recorded the following obstetric and neonatal data: type of red cell alloimmunization, number of IUTs, gestational

age at birth, birth weight, small for gestational age (SGA, defined as below the 10th percentile according to Kloosterman et al.[11]), presence of hydrops at birth, hemoglobin (Hb) level, reticulocyte count, iron status (serum iron, ferritin, transferrin and total iron binding capacity (TIBC)), liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γGT) and lactate dehydrogenase (LDH)) and total serum bilirubin (TSB) and conjugated bilirubin levels at birth. We also recorded duration of phototherapy, number of exchange transfusions (ETs), number of top-up transfusions and days after birth until the last top-up transfusion was given. In addition, we recorded whether infants had sepsis during admission. We prospectively collected data on iron status, bilirubin levels, liver enzymes (measured monthly) and Hb level and reticulocytes (measured weekly) up to three months of age. We collected these data and the number of top-up transfusions received during the first 3 months of life and Hb levels prior to the top-up transfusions through correspondence with the clinical chemistry and/or transfusion laboratories of the outpatient clinics. A uniform transfusion trigger according to our transfusion guidelines was followed. After initial discharge from the LUMC, top-up transfusions were performed when Hb levels fall below 8.0 g/dL or at higher levels (<9.8 g/dL) if clinical symptoms of anemia (lethargy, feeding difficulties or failure to thrive) were present. Iron supplementation was not used; however folic acid (50 µg/day) was administered orally in all infants during the first three months of life.

At birth, iron overload is defined as a serum ferritin level above the 95th percentile and iron deficiency as a serum ferritin level below the 5th percentile according to a recent study of Siddappa et al.[8] In their study, the 95th percentile for term infants (≥37 weeks of gestation) was 309 µg/L and for preterm infants (<37 weeks of gestation) 267 µg/L. The 5th percentile was 40 µg/L for term infants and 35 µg/L for preterm infants.[8] From the first day of life until the age of three months, iron overload and iron deficiency were defined as a ferritin concentration above the 97.5th percentile (775 µg/L) and below the 2.5th percentile (40 µg/L) respectively, according to a study by Soldin et al.[12]

To enhance specificity of high ferritin levels in diagnosing iron overload, transferrin saturation (TSAT) is also calculated (serum iron divided by TIBC and multiplied by 100) in infants with iron overload based on the abovementioned definitions. Iron overload is suggested by a TSAT level of >60% based on the 95th percentile measured in premature newborns of 35-36 weeks of gestation by Lackmann et al.[13]

Cholestasis is defined as a conjugated serum bilirubin concentration above 1.0 mg/dL if the total serum bilirubin level is less than 5 mg/dL, or a value of conjugated bilirubin that represents more than 20% of the total bilirubin if the total bilirubin is greater than 5 mg/dL.[14]

Data are reported as means and standard deviations (SD) or as medians and interquartile ranges (IQR). Statistical analysis was performed using Student-t test and Mann-Whitney test for continuous variables and Chi-square and Fisher's-exact test for categorical variables. A p-value <0.05 was considered to indicate statistical significance. The associations between iron status parameters and top-up transfusions were examined by Spearman correlation coefficients. Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

During the study period, 35 neonates with HDFN due to red cell alloimmunization were admitted to our nursery. Characteristics of included patients are summarized in Table 1. Antibody specificities of included patients were D (n=19), Kell (n=5), E (n=4), c (n=1), D,C (n=1), C,D (n=1), C,E (n=1), D,Wr[a] (n=1), Kell,c (n=1) and D,C,E (n=1). Six percent (2/35) of infants were SGA, of whom one was treated with IUT. Eighty percent (28/35) of infants received one or more top-up transfusions and 71% (20/28) received their first top-up transfusion within one month after birth.

Table 1. Characteristics of study patients

	n = 35
Neonates treated with IUT, n (%)	22 (63)
Number of IUTs [a]	2 (1-3.25, 1-5)
Gestational age at first IUT, weeks [a]	29.8 (23.8-31.2, 18.29-34.29)
Gestational age at birth, weeks [a]	36 (35-37,33-38)
SGA, n (%)	2 (6)
Birth weight, kg [b]	2.88 ± 0.53
Male, n (%)	20 (57)
Neonates treated with phototherapy, n (%)	35 (100%)
Days of phototherapy per infants [b]	4.49 ± 1.93
Neonates treated with ET, n (%)	9 (25.7)
Number of ETs [a]	1 (1-1.5,1-2)
Neonates treated with top-up transfusions, n (%)	28 (80)
Number of top-up transfusions [a]	2 (1-3,1-6)
Days after birth until first top-up transfusion [a]	17 (3-31, 0-42)
Days after birth until last top-up transfusion [a]	34.5 (25.5-60.5, 0-79)

[a] Value given as median (IQR, range)

[b] Value given as mean ± SD

Table 2 provides an overview of the course of iron status, bilirubin levels (total and conjugated) and liver enzymes measured at birth, at one month, at two months and three months after birth. Evaluation of all parameters at each time point was often incomplete. Median ferritin level was 442 µg/L at birth (T=0), 763 µg/L at one month (T=1), 630 µg/L at two months (T=2) and 417 µg/L at three months of age (T=3). Minimum and maximum ferritin levels were 103 µg/L and 1211 µg/L at T=0, 154 µg/L and 2700 µg/L at T=1, 70 µg/L and 1504 µg/L at T=2 and 33 µg/L and 1100 µg/L at T=3.

Table 2. Course of iron status, bilirubin, liver enzymes and red blood cell indices in infants with alloimmune HDFN

	T=0 (at birth)	**T= 1 (at one month)**	**T=2 (at two months)**	**T=3 (at three months)**
Ferritin level, µg/L[a]	442 (242-710)	763 (413-936)	630 (311-935)	417 (184-704)
Iron level, µmol/L[b]	34.0 ± 7.5	20.4 ± 7.0	14.2 ± 7.7	12.7 ± 6.1
TIBC, µmol/L[c]	33 (29.3-37)	36 (30-40)	48 (40-53)	n=2: 44 and 49
Transferrin, g/L[d]	1.61 (1.45-1.86)	1.50 (1.30-1.60)	1.70 (1.42-1.94)	2 (1.70-2.24)
TSB level, µmol/L[e]	77 (62-117)	28 (13-52)	12 (7-16)	8 (5.3-10)
Conjugated bilirubin level, µmol/L[f]	10 (8-12)	8 (4.3-16)	6 (1-12)	2 (0.5-12.5)
AST, U/L[g]	34 (26-48)	33 (22-43)	39 (21-51)	39 (26-56.5)
ALT, U/L[h]	12 (8.5-15)	24 (17-28.5)	33 (25-66)	39.5 (24-56)
γGT, U/L[i]	126 (73.5-212)	80 (51-116)	51.5 (38.5-83.3)	29 (21.5-40.0)
LDH, U/L[j]	434 (343.5-635.5)	253 (233.5-309.5)	257.5 (206.5-291)	236 (196-290)
Hb level, g/dL[k]	12.9 (11.0-15.8)	8.5 (7.9-9.5)	8.9 (8.2-10.0)	9.8 (9.3-10.8)
Reticulocyte count, ‰[l]	57 (16-101)	4.5 (2-22.8)	27 (13.3-41.1)	22.8 (20.2-35.7)

Values are given as mean ± SD or median (IQR). Hb = hemoglobin.

[a] Measured in 33, 20, 12 and 11 infants at T = 0, 1, 2, and 3, respectively. [b] Measured in 31, 18, 12 and 10 infants at T = 0, 1, 2, and 3, respectively. [c] Measured in 32, 7, 5 and 2 infants at T = 0, 1, 2, and 3, respectively [d] Measured in 32, 19, 11 and 9 infants at T = 0, 1, 2, and 3, respectively. [e] Measured in 35, 23, 11 and 9 infants at T = 0, 1, 2, and 3, respectively. [f] Measured in 31, 19, 5 and 5 infants at T = 0, 1, 2, and 3, respectively. [g] Measured in 33, 19, 12 and 9 infants at T = 0, 1, 2, and 3, respectively. [h] Measured in 33, 21, 11 and 10 infants at T = 0, 1, 2, and 3, respectively. [i] Measured in 34, 23, 10 and 10 infants at T = 0, 1, 2, and 3, respectively. [j] Measured in 29, 17, 12 and 9 infants at T = 0, 1, 2, and 3, respectively. [k] Measured in 35, 29, 22 and 15 infants at T = 0, 1, 2, and 3, respectively. [l] Measured in 35, 22, 18 and 14 infants at T = 0, 1, 2, and 3, respectively.

Table 3. Influence of intrauterine transfusions on iron status, liver enzymes and red cell indices at birth and at one month after birth

	T=0 (at birth)			T= 1 (at one month)		
	IUT	**No IUT**	**P- value**	**IUT**	**No IUT**	**P-value**
Ferritin level, µg/L[a]	598 ± 249	270 ± 111	< 0.001	815 (720-982)	434 (315-863)	0.181
Iron level, µmol/L[b]	33 (30.5-37)	29 (26-41.5)	0.367	22.1 ± 5.7	17.8 ± 8.4	0.216
Transferrin, g/L[c]	1.65 ± 0.33	1.71 ± 0.29	0.606	1.46 ± 0.38	1.55 ± 0.14	0.577
TSB, µmol/L[d]	101.5 (60-135.5)	73 (65-110)	0.389	29 (19.5-82)	20 (12-47)	0.280
Conjugated bilirubin, µmol/L[e]	10 (8-13.25)	8 (7.5-9.5)	0.070	8 (4.075-25)	8 (7-11)	0.902
LDH, U/L[f]	434 (332-719)	450 (344-522)	0.830	238 (234-264)	306 (223-379)	0.481
AST, U/L[g]	27.5 (24.8-48.8)	39 (32-50)	0.233	32.5 (22.3-41.5)	35 (18-43)	0.711
ALT, U/L[h]	11.5 (8-14.3)	14 (9-18)	0.355	24 (17-27.5)	25 (12.8-35.0)	0.547
γGT, U/L[i]	134.5 (73.5-207.5)	97.5 (71.5-238.8)	0.958	80 (52-116)	82.5 (36.8-180.3)	0.776
Hb, g/dL[j]	12.0 (10.5-13.5)	14.5 (12.6-16.9)	0.026	8.5 ± 1.4	8.9 ± 1.3	0.469
Reticulocyte count, ‰[k]	24 (2-142)	73 (59.5-124)	0.004	4 (2-20.5)	15.9 (2.75-34)	0.231

Values are given as mean ± SD or median (IQR). Hb = hemoglobin.

[a] Measured in 21 (IUT group) and 12 infants (no IUT group) at birth and in 12 (IUT group) and 8 (no IUT group) infants at one month.

[b] Measured in 21 (IUT group) and 10 infants (no IUT group) at birth and in 11 (IUT group) and 7 (no IUT group) infants at one month.

[c] Measured in 21 (IUT group) and 11 infants (no IUT group) at birth and in 11 (IUT group) and 8 (no IUT group) infants at one month.

[d] Measured in 22 (IUT group) and 13 infants (no IUT group) at birth and in 12 (IUT group) and 11 (no IUT group) infants at one month.

[e] Measured in 22 (IUT group) and 9 infants (no IUT group) at birth and in 12 (IUT group) and 7 (no IUT group) infants at one month.

[f] Measured in 21 (IUT group) and 8 infants (no IUT group) at birth and in 9 (IUT group) and 8 (no IUT group) infants at one month.

[g] Measured in 22 (IUT group) and 11 infants (no IUT group) at birth and in 12 (IUT group) and 7 (no IUT group) infants at one month.

[h] Measured in 22 (IUT group) and 11 infants (no IUT group) at birth and in 13 (IUT group) and 8 (no IUT group) infants at one month.

[i] Measured in 22 (IUT group) and 12 infants (no IUT group) at birth and in 15 (IUT group) and 8 (no IUT group) infants at one month.

[j] Measured in 22 (IUT group) and 13 infants (no IUT group) at birth and in 19 (IUT group) and 10 (no IUT group) infants at one month.

[k] Measured in 22 (IUT group) and 13 infants (no IUT group) at birth and in 16 (IUT group) and 6 (no IUT group) infants at one month.

Table 3 shows the comparison of parameters measured at birth and at one month after birth between infants treated with or without IUT. Ferritin level at birth was significantly higher in infants treated with IUT than in infants not treated with IUT (mean ± SD: 598 ± 249 and 270 ± 111, respectively, p=<0.001). Hb level and reticulocyte count at birth were significantly lower in infants treated with IUT (median (IQR): 7.45 (6.5-8.4) and 9 (7.8-10.5), p=0.026 and 24 (2-142) and 73 (59.5-124), p=0.004, respectively). At one month of age, no significant differences were found between the groups treated with or without IUT.

Seventy percent of neonates (23/33) had iron overload at birth and 83% (19/23) of these infants had been treated with IUT. Twenty percent of neonates (2/10) without iron overload at birth had been treated with IUT. Iron overload was present in 10/20 (50%) infants at one month, in 5/12 (42%) infants at two months and 2/11 (18%) infants at three months of age. In all neonates with iron overload at birth TSAT was >60% (mean of 86%), except one neonate with a TSAT of 44%. The TSAT was measured in only two of ten neonates with iron overload at one month of age (54% and 73%), in only one of five neonates with iron overload at the age of two months (24%) and in none of the neonates with iron overload at the age of three months.

None of the infants had iron deficiency at birth. However, one term infant gradually developed iron deficiency after birth (ferritin level at birth: 145 µg/L, at T=1: 154 µg/L, at T=2: 70 µg/L, at T=3: 33 µg/L). This male infant with Rh D alloimmune HDFN had received one IUT and no top-up transfusions. Serum iron level at 3 months was 9 µmol/L and serum transferrin level 2.40 g/L.

Five of 35 neonates (14%) had cholestasis during admission and those five neonates all had iron overload at birth with ferritin levels varying from 322 µg/L to 1211 µg/L. Four of five cholestatic neonates had Rh D HDFN, one had Kell HDFN, and four of these five had been treated with IUT.

Only one infant (treated with ET) had signs of neonatal sepsis during admission with a positive blood culture for Staphylococcus Aureus. After exclusion of this infant, ferritin level at birth remained significantly higher in infants treated with IUT than those not treated with IUT (598 ± 249 and 269 ± 116 respectively, p= <0.001).

There were no significant correlations between the number of top-up transfusions and ferritin level, serum iron, TIBC and transferrin measured at one month after birth (data not shown).

In infants who did not receive top-up transfusions, ferritin levels at one month, two months and three months after birth varied between 154-715 (n=4), 70-435 (n=3) and 33-122 (n=2), respectively.

Discussion

This study shows the course of iron status after birth until the age of three months in infants with alloimmune HDFN. Based on our definitions, we found that 70% had iron overload and none had iron deficiency at birth. After birth, the incidence of iron overload gradually decreased from 50% at the age of one month to 18% at the age of three months.

The high ferritin levels at birth in our study group are consistent with a previous small study by Berger et al. They found a mean ferritin level of 372 µg/L at birth in 14 neonates with Rh D hemolytic disease of whom 9 (64%) were treated with IUT. In addition, the mean serum iron level at birth and the number of infants treated with IUT in our study are also comparable with their study.[3] IUTs are associated with increased fetal ferritin levels, but ferritin levels were also increased in fetuses with Rh alloimmune hemolytic disease before the first IUT in a study by Nasrat et al.[4] The significantly higher ferritin levels at birth in IUT treated neonates in our study are consistent with the findings of Nasrat et al. In a study by Aygun et al. cord blood ferritin levels in infants with Rh D HDFN were also significantly higher than levels in a control group. However, they found no difference in ferritin levels between neonates with Rh D HDFN treated with or without IUT.[2]

There is a number of case reports published on the risk of severe iron overload, diagnosed by liver biopsies, following IUTs for Rh HDFN.[6,7,15,16] These infants were all born at 33 or 34 weeks of gestation and received 2-5 IUTs and several postnatal transfusions. Their serum ferritin levels ranged from 2479 to 28800 µg/L.[6,7,15,16]

In addition to transfusions for alloimmune HDFN, the hemolysis itself can also contribute to iron overload in alloimmune HDFN. Abbas et al. measured maternal and fetal serum ferritin concentrations at 18-38 weeks of gestation in 40 red alloimmunized pregnancies and compared them to those of normal pregnancies. They found that serum ferritin concentrations are increased in the fetus and decreased in the mother. Their explanation for these findings was enhanced transplacental iron transfer despite fetal iron overload due to the extravascular hemolysis similar to increased iron absorption from the gastrointestinal tract in certain types of postnatal hemolytic anemia.[5]

Follow up ferritin levels are described in several studies in term and preterm neonates without alloimmune HDFN.[17-19] However, no studies report ferritin levels for neonates with alloimmune HDFN after birth. In a study by Mukhopadhyay et al. the median ferritin level in 50 term (≥37 weeks of gestation) appropriate for gestational age neonates at birth was 141 µg/L and mean level at one month of age was 226 µg/L.[19] In our study group, ferritin levels at one month after birth were higher than in their study group of neonates without alloimmune HDFN. However, in their study none of the infants received a red blood cell

transfusion in contrast to our study in which 80% of infants were transfused postnatally of whom 71% were transfused within one month after birth.[19] Another difference between both studies was the gestational age of participants. In our study also premature infants were included. Because most of fetal iron is transported from mother to fetus in the third trimester of pregnancy, prematurely born infants have lower iron stores at birth.[8] Schiza et al. showed that serum ferritin levels decreased from the age of 2 weeks to 6 months and were stable thereafter in late premature infants (34-36 weeks of gestation). At the chronological age of three months they found a mean ferritin level of 70 (± 68) µg/L. Soldin et al. summarized serum ferritin levels in 800 in- and outpatient participants. Reference intervals for ferritin levels in males and females of 0-90 days of age were 40-775 and 79-501 µg/L, respectively.[12]

In recent literature, a ferritin level <12 µg/L is used for the definition of iron deficiency during the first year of life.[17,20] Based on that definition, no cases of iron deficiency were present until three months of age in our study group. Based on the lower reference range (40 µg/L) in the study of Soldin et al., only one neonate met the definition of iron deficiency. This male term infant with Rh D HDFN received one IUT but no postnatal top-up transfusions. Whether this neonate may have benefited from iron supplementation is not clear.

The incidence of cholestasis in this study is similar as reported in a previous study form our group.[21] In the previous report IUT and Rh D type of alloimmune HDFN were identified as risk factors for cholestasis. In the present study, the majority of cholestatic neonates was also treated with IUT and had Rh D type of alloimmunization. In addition, all five cholestatic neonates had iron overload at birth. Although the numbers are small, these results are consistent with the previously published hypothesis that iron overload can cause cholestasis in neonates with alloimmune HDFN.[21]

This is the first study describing follow up data on iron status in neonates with alloimmune hemolytic disease exclusively. However, conclusions are limited by the relative small numbers of participants. In addition, iron overload is difficult to diagnose in neonates and ferritin levels could have been influenced by infection, liver damage, ineffective erythropoiesis, and ascorbate deficiency.[9,10] Follow up studies should also take into account factors that can influence neonatal iron status such as maternal diabetes, pregnancy induced hypertension, maternal smoking, severe maternal iron deficiency, intrauterine growth restriction and type of feeding of the infants (formula or breastfeeding).[8]

In conclusion, iron deficiency in the first three months of life is very rare among infants with alloimmune HDFN and does not occur in infants who received top-up transfusions postnatally. On the contrary, iron overload occurs in 70% of neonates with alloimmune

HDFN at birth, 50% at the age of one month and 18% at the age of three months. Therefore, we advise to measure iron status and we discourage the use of iron supplementation in the first three months of life in neonates with alloimmune HDFN. Hemolysis and intrauterine and postnatal transfusions probably both contribute to the high incidence of iron overload in alloimmune HDFN. Further studies are required to confirm our findings and to study the long term effects of iron overload in neonates with alloimmune HDFN.

References

1. Rath ME, Smits-Wintjens VE, Walther FJ, Lopriore E. Hematological morbidity and management in neonates with hemolytic disease due to red cell alloimmunization. Early Hum.Dev. 2011 Sep;87(9):583-588.
2. Aygun C, Tekinalp G, Gurgey A. Increased fetal iron load in rhesus hemolytic disease. Pediatr. Hematol.Oncol. 2004 Jun;21(4):329-333.
3. 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 Apr 21;335(8695):933-936.
4. Nasrat HA, Nicolini U, Nicolaidis P, Letsky EA, Gau G, Rodeck CH. The effect of intrauterine intravascular blood transfusion on iron metabolism in fetuses with Rh alloimmunization. Obstet. Gynecol. 1991 Apr;77(4):558-562.
5. Abbas A, Nicolaides K. Fetal serum ferritin and cobalamin in red blood cell isoimmunisation. Fetal Diagn.Ther. 1995 Sep;10(5):297-300.
6. Lasker MR, Eddleman K, Toor AH. Neonatal hepatitis and excessive hepatic iron deposition following intrauterine blood transfusion. Am.J.Perinatol. 1995 Jan;12(1):14-17.
7. Sreenan C, Idikio HA, Osiovich H. Successful chelation therapy in a case of neonatal iron overload following intravascular intrauterine transfusion. J.Perinatol. 2000 Dec;20(8 Pt 1):509-512.
8. 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(2):73-82.
9. Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. Int.J.Hematol. 2008 Jul;88(1):7-15.
10. Brittenham GM, Badman DG. Noninvasive measurement of iron: report of an NIDDK workshop. Blood 2003 Jan 1;101(1):15-19.
11. Kloosterman GJ. [Intrauterine growth and intrauterine growth curves]. Ned.Tijdschr.Verloskd. Gynaecol. 1969 Oct;69(5):349-365.
12. Soldin OP, Bierbower LH, Choi JJ, Choi JJ, Thompson-Hoffman S, Soldin SJ. Serum iron, ferritin, transferrin, total iron binding capacity, hs-CRP, LDL cholesterol and magnesium in children; new reference intervals using the Dade Dimension Clinical Chemistry System. Clin.Chim.Acta 2004 Apr;342(1-2):211-217.
13. Lackmann GM, Schnieder C, Bohner J. Gestational age-dependent reference values for iron and selected proteins of iron metabolism in serum of premature human neonates. Biol.Neonate 1998;74(3):208-213.
14. Moyer V, Freese DK, Whitington PF, Olson AD, Brewer F, Colletti RB, Heyman MB. Guideline for the evaluation of cholestatic jaundice in infants: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J.Pediatr.Gastroenterol.Nutr. 2004 Aug;39(2):115-28.
15. Yalaz M, Bilgin BS, Koroglu OA, Ay Y, Arikan C, Sagol S, Akisu M, Kultursay N. Desferrioxamine treatment of iron overload secondary to RH isoimmunization and intrauterine transfusion in a newborn infant. Eur.J.Pediatr. 2011 Nov;170(11):1457-60.

16. Yilmaz S, Duman N, Ozer E, Kavas N, Oren H, Demircioglu F, Kumral A, Ozkan H, Irken G, Ozer E. A case of rhesus hemolytic disease with hemophagocytosis and severe iron overload due to multiple transfusions. J.Pediatr.Hematol.Oncol. 2006 May;28(5):290-292.
17. Schiza V, Giapros V, Pantou K, Theocharis P, Challa A, Andronikou S. Serum transferrin receptor, ferritin, and reticulocyte maturity indices during the first year of life in 'large' preterm infants. Eur.J.Haematol. 2007 Nov;79(5):439-446.
18. Makela E, Takala TI, Suominen P, Matomaki J, Salmi TT, Rajamaki A, Lapinleimu H, Lehtonen L, Irjala K, Lahteenmaki PM. Hematological parameters in preterm infants from birth to 16 weeks of age with reference to iron balance. Clin.Chem.Lab Med. 2008;46(4):551-557.
19. Mukhopadhyay K, Yadav RK, Kishore SS, Garewal G, Jain V, Narang A. Iron status at birth and at 4 weeks in term small-for-gestation infants in comparison with appropriate-for-gestation infants. J.Matern.Fetal Neonatal Med. 2011 Jul;24(7):886-890.
20. Franz AR, Mihatsch WA, Sander S, Kron M, Pohlandt F. Prospective randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams. Pediatrics 2000 Oct;106(4):700-706.
21. Smits-Wintjens VE, 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(4):306-310.

Chapter 12

Summary and general discussion

Summary and general discussion

This thesis investigated the management and outcome of neonates with hemolytic disease of the fetus and newborn (HDFN) due to red cell alloimmunization. The focus of this thesis was mainly on the hematological complications and transfusion practices rather than on the management and treatment options of hyperbilirubinemia.
Hematological complications of HDFN include (early and late) anemia, thrombocytopenia, leukocytopenia, coagulation disorders and iron overload. An overview of the literature on these hematological complications and its treatment options in neonates with HDFN due to red cell alloimmunization was given in **Chapter 2**.

Anemia necessitating top-up transfusions in red cell alloimmune hemolytic disease can be present in the first three months of life. Early onset anemia (within 7 days after birth) is mainly caused by antibody induced hemolysis of red blood cells (RBCs) and characterized by a high bilirubin level and an increased reticulocyte count. Late onset anemia can be subdivided in “late hyporegenerative anemia” characterized by inappropriate erythropoiesis and “late anemia of hemolytic disease” with an active bone marrow and normal or high reticulocyte counts.[1] However, this subdivision is theoretical and in practice these forms can coincide.
Severity and duration of anemia can be difficult to predict in individual cases and as a result frequent monitoring of hemoglobin level and reticulocyte count is required. Factors that are associated with a more severe course of anemia include on one hand a high hemolytic potential of the antibodies (which is determined by the strength (titer) and IgG-subclass) and on the other hand the antigen density of the target antigen on fetal/neonatal red cells or erythrocyte precursor cells. Also treatment with intrauterine transfusion (IUT) by the mechanism of suppression of fetal erythropoiesis contributes to neonatal anemia.[2,3] In contrast, exchange transfusion (ET) was associated with less occurrence of late anemia in a study of 36 infants with Rh D HDFN, probably due to the elimination of antibodies from the circulation which reduces hemolysis and anemia.[1]

Exchange transfusions

In **Chapter 3** we studied the influence of ET practices in a large group of near-term neonates with Rh hemolytic disease. In 2005 a more restrictive ET guideline, based on the American Academy of Pediatrics (AAP) guidelines, was implemented at our center. After implementation of that guideline, ET rate decreased considerably from 66% to 26%. The rate of top-up transfusion however was increased from 68% to 81% after guideline change.

In addition, we found a significant negative correlation between the number of ETs and the number of top-up transfusions in those infants treated with IUT. These results suggest that removal of antibodies and replacing Rh positive cells by Rh negative cells through ET is particularly effective in reducing anemia in the more severely affected neonates with high antibody titers.

In 1925 the first successful ET was performed by Hart for the treatment of severe jaundice in a newborn infant.[4] However, it was until the 1940s that exchange transfusions became common practice in the treatment of neonates with HDFN.[5-7] ET has more beneficial effects in addition to removing excess bilirubin. ET also removes part of the antibodies but most importantly, similarly as with IUT, the infants' blood is replaced by red cells that are immunologically compatible with mother and infant, hence not destructed by the action of remaining antibodies. Although ET carries less risk than IUT, ET remains an invasive procedure with a significant risk of side effects. Although reported mortality rates are less than 2%, morbidity rates can be as high as 74% depending on how adverse events are defined and the population in which they are measured.[8-15] Adverse events include cardio-respiratory events (apneas, cardiac arrest, hypo- and hypertension, cardiac rhythm disorders, pulmonary hemorrhage) catheter-related complications (sepsis, malposition, thrombus), those related to the use of blood products (infection, graft versus host reactions), metabolic derangements (acidosis, disturbances of serum calcium, glucose, potassium and sodium) and other serious complications such as necrotizing enterocolitis, and bowel perforation.[8-15] We performed a retrospective study to evaluate the type and rate of ET-associated complications in a large series of neonates with HDFN due to red cell alloimmunization exclusively (**Chapter 4**). Comparison between ET-treated neonates and non-ET-treated neonates showed that ET treatment was independently associated with an increased risk on: clinical and blood-culture positive sepsis (8% versus 1%, OR 8.3, 95% CI 1.7-40.3, p = 0.009), leukocytopenia (88% versus 23%, OR 36.0, 95% CI 17.5-73.8, p = <0.001), severe thrombocytopenia (platelet count $<50 \times 10^9$/L) (63% versus 8%, OR 31.4, 95% CI 14.0-70.4, p = <0.001), hypocalcemia (22% versus 1%, OR 27.4, 95% CI 5.9-126.8) and hypernatremia (8% versus 0%, p = <0.001). Severe symptoms of hypernatremia (seizures) did not occur and neonatal death did also not occur in the group treated with ET. In our center platelet transfusions are given when platelet counts fall below 100×10^9/L before planned ET and $<50 \times 10^9$/L after ET. In the ET-treated group only one infant with severe thrombocytopenia showed an intracerebral bleeding (ICB) on cranial ultrasound. In the non-ET-treated group three neonates had signs of ICB on cranial ultrasound and/or MRI of whom one had severe thrombocytopenia for which he received 4 platelet transfusions. We found no difference in mortality and severe morbidity (ICB) between ET- and non-ET-treated neonates.

Intravenous immunoglobulin

Since ET can be associated with significant procedure-related morbidity, alternatives to prevent ET or to optimize the effect of ET have been investigated. These alternatives include (maternal) phenobarbital, albumin, metalloporphyrins, clofibrate and IVIg.[16] Except for the latter, routine use of these alternatives are not recommended because evidence on the effectiveness is too limited or treatment has been associated with serious adverse effects.[16-19] Although the exact mechanism of IVIg remains unclear, IVIg might reduce the rate of hemolysis in alloimmune HDFN by blockade of Fc-receptors involved in phagocytosis of antibody coated RBCs. In particular in Rh D antagonism, RBCs are destroyed by an antibody-dependent cellular cytotoxic (ADCC) mechanism mediated by Fc-receptor bearing cells of the neonatal reticulo-endothelial system.[20] The 2004 guidelines of the AAP recommend the use of 0.5-1.0 g/kg IVIg in neonates with alloimmune HDFN if total serum bilirubin (TSB) is rising despite intensive phototherapy or if TSB level is within 34-51 µmol/L (2-3 mg/dL) of exchange level.[17] This recommendation was also based on limited evidence. Although results of the Cochrane review in 2002 showed a significant reduction in the need for ET in infants treated with IVIg, the applicability of the results was limited because none of three included studies was of high quality. The Cochrane authors concluded that further well-designed trials were required before routine use of IVIg could be recommended. We therefore performed a double-blind placebo-controlled RCT on the prophylactic use of IVIg in red cell alloimmune HDFN. We demonstrated that IVIg does not reduce the need for and number of ETs and top-up transfusions nor the duration of phototherapy and maximum bilirubin levels (**Chapter 5**).

Hereafter, we updated the Cochrane review (**Chapter 6**) and included fourteen (quasi-) RCTs of which only two were of high quality.[21-35] Meta-analysis of all fourteen trials, comprising 942 infants, showed a reduction in the need for and number of ETs in infants treated with IVIg combined with phototherapy compared to infants treated with phototherapy only. However, analysis of the only two high quality studies showed no reduction in the need for and number of ETs when IVIg was combined with intensive phototherapy. Eight studies measured the effect of IVIg on top-up transfusions for late anemia and found no difference in the need for or number of top-up transfusions. Based on the evidence from high quality trials (without selection bias, performance bias, detection bias, attrition bias and reporting bias) that IVIg is ineffective in preventing ET or top-up transfusion, routine use in alloimmune HDN should be discouraged. However, since there is some evidence that IVIg reduces hemolysis (in laboratory studies)[36,37], future high quality studies are needed to determine whether IVIg has limited role in some infants with alloimmune HDN, for example when ET can not be performed promptly or without unusually high risk.

Alloimmunizations other than Rh D

Anti-Kell and anti-c are after anti-D the most important red cell antibodies causing severe HDFN necessitating IUT.[38] In Kell HDFN antibody titer is not well-correlated with disease severity. Kell antibodies are, after anti-D, the most frequent antibodies causing HDFN.[39] Kell HDFN probably has a different manifestation than Rh HDFN. Because the Kell antigen is expressed earlier on cells of the erythropoietic lineage Kell antibodies can cause destruction of progenitor cells (which are not yet hemoglobinized) in addition to hemolysis of erythrocytes.[40-43] Similar as for Rh HDFN, neonates with Kell hemolytic disease may require top up transfusions up to several months after birth. However, it was not known whether incidence and severity of anemia differs between both types. In **Chapter 7** we therefore compared a group of near-term neonates with Kell HDFN with neonates with Rh D HDFN. We found that neonates with Kell HDFN required less phototherapy (2.4 versus 4.1 days) and ETs (6% versus 62%). The need for top-up transfusions was not significantly different between Kell HDFN and Rh D HDFN (62% versus 72%). The median number of days after birth until first top-up transfusion was also not significantly different (16 days versus 17.5 days). Our results show that infants with Kell HDFN require a different management in the early neonatal period (less phototherapy and less ET because of less formation of bilirubin as breakdown product of hemoglobin), but a similar follow up management as infants with Rh D HDFN.

In **Chapter 8** we performed a similar study and compared postnatal outcome between infants with Rh c HDFN and infants with Rh D HDFN. In this study we excluded cases with multiple clinical significant antibodies because it is known that disease can be more severe when multiple antibodies are present.[44] We found a trend of a slightly more severe antenatal course of Rh c HDFN reflected by an earlier and higher need for IUT (61% versus 41%). Postnatal course was not significantly different between infants with Rh c and Rh D HDFN in terms of phototherapy duration (mean days 4.8 and 4.5), need for ET (50% versus 46%) and need for top-up transfusions (62% versus 78%). Therefore, also for Rh c HDFN a similar follow up management is justified. In this study we also investigated if antibody titer at birth was correlated with postnatal transfusion requirements. For Rh D HDFN it is known that antibody titer is correlated to postnatal anemia.[45] In our study we also found a positive correlation between anti-D titer at birth and need for and number of top-up transfusions. Whether antibody titer in Rh c HDFN is correlated to postnatal transfusion requirements was not known. We found a positive correlation between antibody titer at birth and need for ET.

Treatment of anemia

Treatment of anemia mainly consists of top-up transfusions. International guidelines for top-up transfusions in neonates (with HDFN) are lacking. As a consequence, studies on transfusion requirements are difficult to compare due to differences in transfusion thresholds, volumes, products and different outcome measures. In our center we transfuse 15 ml/kg ABO and Rh type specific, leukocyte-depleted RBCs which are negative for the antigen to which maternal antibodies are directed. Irradiated products are used in infants treated with IUT and/or born <32 weeks of gestation and/or birth weight <1500 gram. Transfusion triggers at our center include a hemoglobin level <5 mmol/L (8 g/dL) or <6 mmol/L (9.6 g/dL) when clinical symptoms of anemia are present (need for extra oxygen, poor feeding, tachycardia, tachypnea).

Supplements to support erythropoiesis which are applied in infants with HDFN include folic acid, iron and vitamin E (tocopherol). Folic acid is essential for proliferation of erythroblasts during their differentiation but also for *de novo* synthesis of the RNA and DNA; hence every growing cell requires folic acid.[46] Gandy and Jacobson et al. demonstrated that 2.5-5 mg/day folic acid supplementation had a beneficial effect on growth, but not on hemoglobin level in infants with alloimmune HDFN.[9,10] Although evidence is lacking, in our center folic acid is supplemented to infants with alloimmune HDFN in a dose of 0.05 mg/day orally during the first three months of life.

Supplementation of iron is not routine practice in our center and the motivation for this is dealt with later in this chapter.

Vitamin E (tocopherol) is occasionally supplemented in neonates with alloimmune HDFN. This antioxidant reduces oxidative stress to the RBC membrane and vitamin E deficiency can therefore lead to a shorter life span of RBCs.[47] Although infants with alloimmune HDFN have lower vitamin E levels compared to healthy controls, administration of large amount of vitamin E analogue in three neonates with Rh D HDFN showed no beneficial effects.[48,49] In our center, vitamin E is not supplemented to neonates with alloimmune HDFN.

Recombinant human erythropoietin (rhEPO) is used, often combined with supplements to support erythropoiesis, to treat (late) anemia in HDFN due to red cell alloimmunization. In Table 2 of **Chapter 2** an overview is provided of studies/case reports of administration of rhEPO in infants with alloimmune HDFN. Only one small randomized placebo controlled trial has been performed in 20 neonates (≥35 weeks' gestation) with Rh HDFN showing a significantly lower number of top-up transfusions in the rhEPO treated group.[50]

Associated hematological morbidity

Thrombocytopenia and coagulation disorders

Thrombocytopenia was first reported in 1955 in three neonates with alloimmune HDFN.[51] In 1970, Nielsen showed that neonates with alloimmune HDFN had a decreasing platelet count and an increasing clot-promoting activity (measured by the thromboplastin activation test in plasma) at birth associated with increase of disease severity.[51] In 1979, a report was published by Hey and Jones on coagulation failure in Rh alloimmunization.[52] They found very low vitamin-K dependent coagulation factor levels in cord blood of seven infants with severe disease probably caused by liver damage in utero. Five severely diseased infants died of pulmonary or cerebral hemorrhages. In addition, they demonstrated a strong correlation between platelet count and hemoglobin level at birth. Infants with a cord blood hemoglobin level of <10 g/dL (6.2 mmol/L) had a platelet count $<150 \times 10^9$/L.[52]

The exact etiology of thrombocytopenia in alloimmune HDFN is still unknown, but several mechanisms have been suggested including increased immunological destruction and/or bilirubin toxicity[53], sequestration of platelets due to hypersplenism[52], dilution due to platelet poor (intrauterine) erythrocyte transfusions[54], increased consumption (disseminated intravascular coagulation)[55] and decreased production. Koenig et al. suggested a decreased platelet production in favor of increased erythrocyte production.[56] In their study group of 20 neonates with Rh HDFN, eleven neonates with hydrops (severe disease) or who were treated with ET (moderate disease) had thrombocytopenia and none of the nine neonates without hydrops or ET had thrombocytopenia. The thrombocytopenic neonates did not have an increased platelet volume. In a severely affected neonate, they also demonstrated that the in vitro maturation of multipotent progenitor cells was altered, with a greater proportion of normoblasts, and fewer neutrofils and megakaryocytes. The significant higher incidence of thrombocytopenia in hydropic (more severely affected) fetuses and the negative correlation between reticulocyte and platelet count that Saade et al. demonstrated, also supports the decreased platelet production theory.[57]

Because IUT has improved significantly over the years, severe hemolytic disease with hydrops has become less common nowadays. In a recent large study (**Chapter 9**) we demonstrated that 26% (94/362) and 6% (20/362) of neonates with alloimmune HDFN had thrombocytopenia (platelet count $<150 \times 10^9$/L) and severe thrombocytopenia (platelet count $<50 \times 10^9$/L) at birth, respectively. Risk factors that were independently associated with thrombocytopenia included treatment with IUT, being small for gestational age and a lower gestational age at birth. Although fetal hydrops was not significant at the 5% level,

the relative low p-value (p = 0.083) is suggestive of a possible independent association with thrombocytopenia at birth. Only one neonate had clinical signs of bleeding at birth. This hydropic premature infant with a platelet count of 53 × 10^9/L at birth had an intraventricular hemorrhage (IVH) grade 2 on day one.
In our center a concentrated platelet transfusion in a dose of 20 × 10^9/kg is given if: 1) platelet count is <20 × 10^9/L in clinically stable neonates; 2) platelet count is <50 × 10^9/L in neonates with a manifest bleeding, those undergoing a procedure with a risk of bleeding and in clinically unstable neonates weighing <1500 grams and of <32 weeks of gestation, 3) platelet count is <100 × 10^9/L before ET (halfway the ET a platelet transfusion is performed). However, the causal relationship between thrombocytopenia and IVH in preterm infants is controversial.[58-60] Moreover, platelet transfusion are also occasionally associated with adverse effects such as transfusion reactions and transmission of infectious agents.[61,62] In addition, the role of platelet transfusions in preventing bleeding is still unclear in neonates.[63]

Cholestasis

Few studies, mostly case-reports, have reported on the occurrence of conjugated hyperbilirubinemia, i.e. cholestasis, in alloimmune HDFN.[64-67] Cholestasis in neonates with alloimmune HDFN has been associated with iron overload due to IUTs.[49,65,68,69] Other suggested etiologic mechanisms include overload of pigment causing stasis and blocking of bile canaliculi; liver necrosis caused by hypoxia due to anemia; and pressure by extramedullary hematopoiesis in the liver caused by anemia leading to damage of intrahepatic canaliculi.[70] In **Chapter 10** we evaluated the incidence, potential risk factors, management and outcome of cholestasis in 313 neonates with alloimmune HDFN due to various anti-RBC antibody specificities. Cholestasis occurred in 13% and was independently associated with IUT treatment and Rh D antibodies. However, 88% of cholestatic neonates had both Rh D HDFN and were treated with IUT, preventing reliable distinction between both risk factors. Extensive tests to rule out other causes of cholestasis were all negative. However, they were not performed in all neonates with cholestasis. In almost half of the patients cholestasis resolved spontaneously within 1 week to 3 months after birth. In the other half of the patients, measurements of bilirubin and liver enzymes were not monitored until they reached normal values. Median maximum ferritin levels during admission were significantly higher in cholestatic neonates than in the non-cholestatic neonates (1191 (range: 489-73000) µg/L and 657 (range: 86-10195) µg/L, p = <0.001). In addition, maximum ferritin level and treatment with IUT were positively correlated (r = 0.565, p = <0.001). One cholestatic patient with severe hyperferritinemia was treated with iron chelation therapy.

Iron overload

Ferritin reflects total body iron stores and age-appropriate levels are used to determine iron deficiency and iron overload. In healthy term newborns median ferritin levels at birth of 134-141 µg/L have been reported.[71,72] Mean ferritin levels at birth have been reported to range from 101 µg/L to 183 µg/L.[73,74] After birth, ferritin levels rise to a mean level of 226-356 µg/L and than decrease progressively over the following months to a mean level around 30 µg/L at the age of 6 months.[71,74,75] Although it is known that ferritin levels in the fetal period and at birth can be highly elevated in neonates with alloimmune HDFN, the course of ferritin levels in the following months after birth was not known.[49,68,69] We therefore prospectively collected data on the iron status of 35 neonates with alloimmune HDFN treated with or without IUT (**Chapter 11**). We found that iron overload occurred in 70% (23/33) of neonates with alloimmune HDFN at birth, 50% (10/20) at the age of one month, 42% (5/12) at the age of two months and 18% (2/11) at the age of three months. We found no cases of iron deficiency at birth and only one term infant had iron deficiency at three months of age based on the for this study defined criterion for iron deficiency (<40 µg/L). Whether this infant, who received one IUT and no postnatal transfusions, would have benefitted from iron supplementation is not clear. In our cohort, none of the infants received iron supplementation. We recommend to measure iron status before starting iron supplementation in infants with alloimmune HDFN because iron deficiency as well as iron overload can have detrimental effects. Iron deficiency and overload have both been associated with neurodevelopmental impairment.[72] Iron overload can also lead to damage to the liver, heart and endocrine organs and increase susceptibility to infection.[72] In adults with hereditary hemochromatosis ferritin levels >1000 µg/L have been associated with increased risk for liver cirrhosis.[76] To assess the long term outcome of iron overload and deficiency in infants with alloimmune HDFN more research is warranted.

Leukocytopenia

As shown in **Chapter 4**, leukocytopenia is a common complication after ET with leukocyte-depleted donor blood. However, Koenig et al. demonstrated that neutropenia was also present before ET in four hydropic neonates with Rh HDFN. They also showed that erythroid progenitors were increased and granulocyte-macrophage progenitors were decreased in two severely affected neonates.[56] Segal et al. described two hydropic neonates with Rh HDFN and neutropenia who were successfully treated with recombinant human granulocyte colony-stimulating factor.[77] These scarce data are insufficient to be conclusive on the incidence and morbidity of neutropenia and its relation with sepsis in alloimmune HDFN.

Conclusion

In conclusion, this thesis focuses on several aspects related to the hematological outcome of infants with HDFN due to RBC alloimmunization, including pathogenesis and management of the disease. The presence of leukocytopenia and thrombocytopenia support the mechanism of suppression of thrombopoiesis and granulopoiesis in favor of the increased erythropoiesis stimulated by anemia and hypoxia. In addition to the problems caused by a shortage of platelets and white and red blood cells, the excess of the RBC metabolites bilirubin and iron also contribute to the morbidity of the disease. Based on our systematic review we can also conclude that there is a lack of high level evidence promoting the use of IVIG. Irrespective of the controversy concerning the use of IVIg, our data show that intensive follow-up is indicated in both Rh D and non-Rh D HDFN and raised awareness about the associated morbidity is of paramount importance.
Although a few questions are answered in this thesis, it yielded much more questions to answer. In the next and final chapter, some of these future research questions are discussed.

References

1. al-Alaiyan S, al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J.Perinat.Med. 1999;27(2):112-5.
2. 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 Jan;198(1):54.
3. Dorn I, Schlenke P, Hartel C. Prolonged anemia in an intrauterine-transfused neonate with Rh-hemolytic disease: no evidence for anti-D-related suppression of erythropoiesis in vitro. Transfusion 2010 May;50(5):1064-70.
4. Hart AP. Familial Icterus Gravis of the New-Born and its Treatment. Can.Med.Assoc.J. 1925 Oct;15(10):1008-11.
5. Diamond LK, Allen FH, Jr., Thomas WO, Jr. Erythroblastosis fetalis. VII. Treatment with exchange transfusion. N.Engl.J.Med. 1951 Jan 11;244(2):39-49.
6. Wallerstein H. Treatment of Severe Erythroblastosis by Simultaneous Removal and Replacement of the Blood of the Newborn Infant. Science 1946 May 10;103(2680):583-4.
7. Wiener AS, Wexler IB, Grundfast TH. Therapy of erythroblastosis fetalis with exchange transfusion. Bull.N.Y.Acad.Med. 1947 Apr;23(4):207-20.
8. Badiee Z. Exchange transfusion in neonatal hyperbilirubinaemia: experience in Isfahan, Iran. Singapore Med.J. 2007 May;48(5):421-3.
9. Davutoglu M, Garipardic M, Guler E, Karabiber H, Erhan D. The etiology of severe neonatal hyperbilirubinemia and complications of exchange transfusion. Turk.J.Pediatr. 2010 Mar;52(2):163-6.
10. Hosseinpour SS, Gharehbaghi MM. Exchange transfusion in severe hyperbilirubinemia: an experience in northwest Iran. Turk.J.Pediatr. 2010 Jul;52(4):367-71.
11. Ip S, Chung M, Kulig J, O'Brien R, Sege R, Glicken S, Maisels MJ, Lau J. An evidence-based review of important issues concerning neonatal hyperbilirubinemia. Pediatrics 2004 Jul;114(1):e130-e153.
12. Jackson JC. Adverse events associated with exchange transfusion in healthy and ill newborns. Pediatrics 1997 May;99(5):E7.
13. Keenan WJ, Novak KK, Sutherland JM, Bryla DA, Fetterly KL. Morbidity and mortality associated with exchange transfusion. Pediatrics 1985 Feb;75(2 Pt 2):417-21.
14. Patra K, Storfer-Isser A, Siner B, Moore J, Hack M. Adverse events associated with neonatal exchange transfusion in the 1990s. J.Pediatr. 2004 May;144(5):626-31.
15. 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 Jul;120(1):27-32.
16. Cuperus FJ, Hafkamp AM, Hulzebos CV, Verkade HJ. Pharmacological therapies for unconjugated hyperbilirubinemia. Curr.Pharm.Des 2009;15(25):2927-38.
17. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004 Jul;114(1):297-316.
18. Suresh GK, Martin CL, Soll RF. Metalloporphyrins for treatment of unconjugated hyperbilirubinemia in neonates. Cochrane.Database.Syst.Rev. 2003;(2):CD004207.

19. 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 Feb;192(2):478-82.
20. Urbaniak SJ. ADCC (K-cell) lysis of human erythrocytes sensitized with rhesus alloantibodies. II. Investigation into the mechanism of lysis. Br.J.Haematol. 1979 Jun;42(2):315-25.
21. Alpay F, Sarici SU, Okutan V, Erdem G, Ozcan O, Gokcay E. High-dose intravenous immunoglobulin therapy in neonatal immune haemolytic jaundice. Acta Paediatr. 1999 Feb;88(2):216-9.
22. Atici A, Satar M, Göðebakan M. Intravenous immunoglobulin therapy for neonatal hyperbilirubinemia due to ABO or Rh incompatibility. [ABO veya Rh Uyuþmazliðinin neden olduðu neonatal hiperbilirübinemide intravenöz immüglobülin tedavisi.]. Çocuk Saðliði ve Hastaliklari Dergisi 1996;39:623-30.
23. Dagoglu T, Ovali F, Samanci N, Bengisu E. High-dose intravenous immunoglobulin therapy for rhesus haemolytic disease. J.Int.Med.Res. 1995 Jul;23(4):264-71.
24. Elalfy MS, Elbarbary NS, Abaza HW. Early intravenous immunoglobin (two-dose regimen) in the management of severe Rh hemolytic disease of newborn--a prospective randomized controlled trial. Eur.J.Pediatr. 2011 Apr;170(4):461-7.
25. Garcia MG, Cordero G, Mucino P, Salinas V, Fernandez LA, Christensen RD. Intravenous Immunoglobulin (IVIG) Administration as a Treatment for Rh Hemolytic Jaundice in Mexico City. Pediatr.Res. 55, 65. 2004.
26. Hematyar M, Zareian M. The effects of intravenous immunoglobulin(IVIG) in hemolytic jaundice of the newborn due to ABO and Rh isoimmunization. Acta Paediatrica 2011 100[Suppl. 463], 71.
27. Miqdad AM, Abdelbasit OB, Shaheed MM, Seidahmed MZ, Abomelha AM, Arcala OP. Intravenous immunoglobulin G (IVIG) therapy for significant hyperbilirubinemia in ABO hemolytic disease of the newborn. J.Matern.Fetal Neonatal Med. 2004 Sep;16(3):163-6.
28. Nasseri F, Mamouri GA, Babaei H. Intravenous immunoglobulin in ABO and Rh hemolytic diseases of newborn. Saudi.Med.J. 2006 Dec;27(12):1827-30.
29. Pishva N, Madani A, Homayoon K. Profylactic intravenous immunoglobulin in neonatal immune hemolytic jaundice. Iranian Journal of Medical Sciences 2000;25:129-33.
30. Rübo J, Wahn V. Influence of high dosage immuno-globulin therapy on hyperbilirubinemia in rhesus-hemolytic disease. A cooperative study. [Kooperative Studie zum Einfluß einer hochdosierten Immunglobulintherapie auf die Hyperbilirubinämie bei Rhesusinkompatibilität.]. Monatsschrift fur Kinderheilkunde 1996;144:516-9.
31. Rübo J, Albrecht K, Lasch P, Laufkotter E, Leititis J, Marsan D, Niemeyer B, Roesler J, Roll C, Roth B, et al. High-dose intravenous immune globulin therapy for hyperbilirubinemia caused by Rh hemolytic disease. J.Pediatr. 1992 Jul;121(1):93-7.
32. Santos MC, Sa C, Gomes Jr 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 2012 Aug 6.doi: 10.1111/j.1537-2995.2012.03827.x.:[Epub ahead of print].
33. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D, Brand A, Lopriore E. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics 2011 Apr;127(4):680-6.
34. Tanyer G, Siklar Z, Dallar Y, Yildirmak Y, Tiras U. Multiple dose IVIG treatment in neonatal immune hemolytic jaundice. J.Trop.Pediatr. 2001 Feb;47(1):50-3.

35. Voto LS, Sexer H, Ferreiro G, Tavosnanska J, Orti J, Mathet ER, Margulies M, Margulies M. Neonatal administration of high-dose intravenous immunoglobulin in rhesus hemolytic disease. J.Perinat.Med. 1995;23(6):443-51.
36. Hammerman C, Vreman HJ, Kaplan M, Stevenson DK. Intravenous immune globulin in neonatal immune hemolytic disease: does it reduce hemolysis? Acta Paediatr. 1996 Nov;85(11):1351-3.
37. Ergaz Z, Gross D, Bar-Oz B, Peleg O, Arad I. Carboxyhemoglobin levels in neonatal immune hemolytic jaundice treated with intravenous gammaglobulin. Vox Sang. 1995;69(2):95-9.
38. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin.Fetal Neonatal Med. 2008 Aug;13(4):207-14.
39. Lee S, Russo D, Redman CM. The Kell blood group system: Kell and XK membrane proteins. Semin. Hematol. 2000 Apr;37(2):113-21.
40. Daniels G, Hadley A, Green CA. Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion 2003 Jan;43(1):115-6.
41. 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 Jul;171(1):247-52.
42. 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 Mar 19;338(12):798-803.
43. Weiner CP, Widness JA. Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anemia. Am.J.Obstet.Gynecol. 1996 Feb;174(2):547-51.
44. Spong CY, Porter AE, Queenan JT. Management of isoimmunization in the presence of multiple maternal antibodies. Am.J.Obstet.Gynecol. 2001 Aug;185(2):481-4.
45. Nordvall M, Dziegiel M, Hegaard HK, Bidstrup M, Jonsbo F, Christensen B, Hedegaard M. Red blood cell antibodies in pregnancy and their clinical consequences: synergistic effects of multiple specificities. Transfusion 2009 Oct;49(10):2070-5.
46. Hoffbrand AV, Weir DG. The history of folic acid. Br.J.Haematol. 2001 Jun;113(3):579-89.
47. Brigelius-Flohe R, Traber MG. Vitamin E: function and metabolism. FASEB J. 1999 Jul;13(10):1145-55.
48. Melhorn DK, Gross S, Lake GA, Leu JA. The hydrogen peroxide fragility test and serum tocopherol level in anemias of various etiologies. Blood 1971 Apr;37(4):438-46.
49. 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 Apr 21;335(8695):933-6.
50. 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 May;39(5):831-4.
51. Nielsen NC. Coagulation and fibrinolysis in rhesus-immunized mothers and their erythroblastotic newborn infants. Acta Obstet.Gynecol.Scand. 1970;49(1):61-9.
52. Hey E, Jones P. Coagulation failure in babies with rhesus isoimmunization. Br.J.Haematol. 1979 Jul;42(3):441-54.
53. Ekert H, Mathew RY. Platelet counts and plasma fibrinogen levels in erythroblastosis foetalis. Med.J.Aust. 1967 Nov 4;2(19):844-6.
54. Vietor HE, Klumper F, Meerman RJ, Brand A, Kanhai HH. Intrauterine transfusions influence fetal leukocyte counts and subsets. Prenat.Diagn. 1998 Apr;18(4):325-31.
55. Chessells JM, Wigglesworth JS. Haemostatic failure in babies with rhesus isoimmunization. Arch. Dis.Child 1971 Feb;46(245):38-45.

56. Koenig JM, Christensen RD. Neutropenia and thrombocytopenia in infants with Rh hemolytic disease. J.Pediatr. 1989 Apr;114(4 Pt 1):625-31.
57. Saade GR, Moise KJ, Jr., Copel JA, Belfort MA, Carpenter RJ, Jr. Fetal platelet counts correlate with the severity of the anemia in red-cell alloimmunization. Obstet.Gynecol. 1993 Dec;82(6):987-91.
58. Von Lindern JS, van den Bruele T, Lopriore E, Walther FJ. Thrombocytopenia in neonates and the risk of intraventricular hemorrhage: a retrospective cohort study. BMC.Pediatr. 2011;11:16.
59. Von Lindern JS, Hulzebos CV, Bos AF, Brand A, Walther FJ, Lopriore E. Thrombocytopaenia and intraventricular haemorrhage in very premature infants: a tale of two cities. Arch.Dis.Child Fetal Neonatal Ed 2012 Sep;97(5):F348-F352.
60. Setzer ES, Webb IB, Wassenaar JW, Reeder JD, Mehta PS, Eitzman DV. Platelet dysfunction and coagulopathy in intraventricular hemorrhage in the premature infant. J.Pediatr. 1982 Apr;100(4):599-605.
61. Blajchman MA. Bacterial contamination of cellular blood components: risks, sources and control. Vox Sang. 2004 Jul;87 Suppl1:98-103.
62. Kluter H, Bubel S, Kirchner H, Wilhelm D. Febrile and allergic transfusion reactions after the transfusion of white cell-poor platelet preparations. Transfusion 1999 Nov;39(11-12):1179-84.
63. Muthukumar P, Venkatesh V, Curley A, Kahan BC, Choo L, Ballard S, Clarke P, Watts T, Roberts I, Stanworth S. Severe thrombocytopenia and patterns of bleeding in neonates: results from a prospective observational study and implications for use of platelet transfusions. Transfus.Med. 2012 Oct;22(5):338-43.
64. Dunn PM. Obstructive Jaundice and Haemolytic Disease of the Newborn. Arch.Dis.Child 1963 Feb;38(197):54-61.
65. Lasker MR, Eddleman K, Toor AH. Neonatal hepatitis and excessive hepatic iron deposition following intrauterine blood transfusion. Am.J.Perinatol. 1995 Jan;12(1):14-7.
66. Perez EM, Cooper TR, Moise AA, Ferry GD, Weisman LE. Treatment of obstructive jaundice in erythroblastosis fetalis with ursodeoxycholic acid (UDCA): a case report. J.Perinatol. 1998 Jul;18(4):317-9.
67. Yilmaz S, Duman N, Ozer E, Kavas N, Oren H, Demircioglu F, Kumral A, Ozkan H, Irken G, Ozer E. A case of rhesus hemolytic disease with hemophagocytosis and severe iron overload due to multiple transfusions. J.Pediatr.Hematol.Oncol. 2006 May;28(5):290-2.
68. Aygun C, Tekinalp G, Gurgey A. Increased fetal iron load in rhesus hemolytic disease. Pediatr. Hematol.Oncol. 2004 Jun;21(4):329-33.
69. Nasrat HA, Nicolini U, Nicolaidis P, Letsky EA, Gau G, Rodeck CH. The effect of intrauterine intravascular blood transfusion on iron metabolism in fetuses with Rh alloimmunization. Obstet. Gynecol. 1991 Apr;77(4):558-62.
70. Sivan Y, Merlob P, Nutman J, Reisner SH. Direct hyperbilirubinemia complicating ABO hemolytic disease of the newborn. Clin.Pediatr.(Phila) 1983 Aug;22(8):537-8.
71. Mukhopadhyay K, Yadav RK, Kishore SS, Garewal G, Jain V, Narang A. Iron status at birth and at 4 weeks in term small-for-gestation infants in comparison with appropriate-for-gestation infants. J.Matern.Fetal Neonatal Med. 2011 Jul;24(7):886-90.
72. 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(2):73-82.
73. Hussain MA, Gaafar TH, Laulicht M, Hoffebrand AV. Relation of maternal and cord blood serum ferritin. Arch.Dis.Child 1977 Oct;52(10):782-4.

74. Siimes MA, Addiego JE, Jr., Dallman PR. Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. Blood 1974 Apr;43(4):581-90.
75. Schiza V, Giapros V, Pantou K, Theocharis P, Challa A, Andronikou S. Serum transferrin receptor, ferritin, and reticulocyte maturity indices during the first year of life in 'large' preterm infants. Eur.J.Haematol. 2007 Nov;79(5):439-46.
76. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, McLaren CE, Bahlo M, Nisselle AE, Vulpe CD, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N.Engl.J.Med. 2008 Jan 17;358(3):221-30.
77. Segal N, Leibovitz E, Juster-Reicher A, Even-Tov S, Mogilner B, Barak Y. Neutropenia complicating Rh-hydrops fetalis: the effect of treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF). Pediatr.Hematol.Oncol. 1998 Mar;15(2):193-7.

Chapter 13

Future perspectives

Future perspectives

In 1932 Diamond et al. reported that hemolysis of fetal and neonatal red blood cells (RBCs) resulted in extramedullary hematopoiesis causing hepatosplenomegaly and a high proportion of erythroblasts in the circulation.[1] Since this description of the pathophysiologic mechanism of alloimmune HDFN, then called erythroblastosis fetalis, numerous discoveries in prenatal and postnatal care have led to a significant decrease in perinatal morbidity and mortality.[2] However, currently there are still several aspects of the pathogenesis, management and outcome of alloimmune HDFN that require further investigation.

Pathogenesis

The cause of late hyporegenerative anemia is still unclear. The following mechanisms have been suggested: intramedullary destruction of RBC precursors and of circulating reticulocytes by RBC antibodies, bone marrow suppression from IUTs, and inappropriate low erythropoietin production for the degree of anemia. [3-6] Burk et al. showed anti-D antibody bound to Rh D positive erythrocytes in the bone marrow in an infant with a high persistent antibody titer.[7] When antibody titer was fallen to 1:64 at eleven weeks, the first evidence of peripheral reticulocyte survival was observed.[7] Late hyporegenerative anemia was already described before the introduction of IUT in 1963 and thereafter was also observed in infants who were not treated with IUT.[6] Dallacasa et al. reported that reticulocyte counts, hemoglobin and erythropoietin (EPO) levels were similar in IUT and non-IUT treated neonates with Rh D alloimmune HDFN.[8] In contrast, in a study by De Boer et al. the percentage of neonates requiring top-up transfusions was significantly higher for IUT-treated neonates than non-IUT-treated neonates with Rh HDFN.[9] Studies that measured EPO-levels conclude differently on whether EPO-levels are appropriate for the degree of anemia or not.[3,5,6] Several small studies and casuistic reports suggest that neonates with alloimmune HDFN may benefit from treatment with recombinant human erythropoietin (rhEPO) to reduce the risk of delayed anemia and subsequent need for top-up transfusions.[10-15] However, other authors found that rhEPO may be less effective than expected.[8,16] Pessler et al. suggested that rhEPO might be ineffective when antibody titers are high.[16] This theory from Pessler sounds plausible since RhEPO in these neonates will produce erythrocytes that can be covered by circulating antibodies and subsequently destructed.

Future randomized controlled trials on the effectiveness of rhEPO in preventing late anemia should be stratified for antibody titer, hemoglobin levels, and gestational age.

Management

Antenatal period

Several pharmacological antenatal management options for alloimmune HDFN deserve further study, including phenobarbital, IVIg and corticosteroids.

Phenobarbital

Antenatal maternal administration of phenobarbital (30 mg 3 times a day for 7-10 days) significantly reduced the need for ET in neonates with HDFN due to RBC alloimmunization in a recent retrospective study by Trevett et al.[17] In neonates with and without antenatal maternal administration of phenobarbital, ET was performed in 9% and 52%, respectively (p=<0.01). Phenobarbital has been shown to improve the capability of the neonatal liver to conjugate and eliminate bilirubin. Because of the immaturity of the hepatic conjugation pathways, premature infants are at greater risk of requiring ET for hyperbilirubinemia. Mainly premature infants were included in the study by Trevett et al. (mean gestational age in ET and no-ET group 33.0 and 35.4 weeks, respectively). Although the effect of phenobarbital remained significant in reducing the need for ET after Trevett et al. adjusted for gestational age, antenatal maternal administration of phenobarbital might be more effective in premature infants with alloimmune HDFN.[17]

IVIg

Several case series and retrospective studies show that antenatal maternal administration of IVIg improves perinatal outcome in Rh D and Kell HDFN.[18-30] Only Chitkara et al. concluded after studying the effect of maternal IVIg in 5 cases with alloimmune HDFN, that this treatment does not appear useful in Rh D HDFN.[31] Suggested mechanism of action of maternal administration of IVIg include: decrease of maternal antibodies by suppression of production or enhanced clearance, competitive inhibition of anti-D transport to the fetus and the inhibition of phagocytosis of fetal antibody-coated red cells by the fetal reticuloendothelial system. Maternal administration of IVIg can not compete with the excellent outcome of IUT treatment but may play an additional role in early hydrops that developed before the 18th week and less eligible for IUT. Fetal administration of IVIG is another option. A great advantage of fetal administration compared with maternal administration is the lower cost since IVIg is dosed per kilogram of body weight. For that reason fetal infusion of IVIg has also been studied.[32-34] Although the case report of Alonso et al. and the case series of Kriplani et al. showed promising results[32,34], a small RCT including 20 fetuses with severe Rh D HDFN by Dooren et al. showed no significant differences in the

transfusion requirements or clinical outcome. However the dose used in this trial was too low to reach reliable conclusions on the effect.[33]

Corticosteroids

Labor is often induced in pregnancies that are complicated by red cell alloimmunization because of the risks of an additional necessary IUT. Late preterm (34 0/7 to 36 6/7 weeks of gestation) and early term (37 0/7 to 38 6/7 weeks of gestation) births are therefore no exception. Recent studies show that late preterm and early term births have a higher rate of morbidity, especially relating to respiratory function and long-term neurodevelopmental outcome, than infants born at 39 weeks of gestation.[35,36] In line with this, it should be evaluated if there is a role for maternal administration of corticosteroids to prevent respiratory complications at birth in infants with alloimmune HDFN. A Cochrane review published in 2006 showed that antenatal corticosteroids significantly reduced the rate of respiratory distress syndrome in neonates (without HDFN) born between 33 0/7 and 34 6/7 weeks of gestation (RR 0.53, 95% CI 0.31 to 0.91, 2 RCTs, 434 infants), but not in neonates born between 35 0/7 and 36 6/7 weeks of gestation (RR 0.61, 95% CI 0.11 to 3.26, 1 RCT, 189 infants).[37] In 2011 a randomized placebo-controlled trial by Porto et al. showed that 12 mg Betamethasone for two consecutive days at 34 0/7 to 36 6/7 weeks of non-HDFN pregnancy does not reduce the incidence of respiratory disorders in neonates.[38] However, the risk of jaundice requiring phototherapy was significantly lower in the group treated with antenatal corticosteroids (RR 0.63, 95% CI 0.44 to 0.91).[38] This finding makes it especially interesting to study the effectiveness of antenatal corticosteroids in neonates with alloimmune HDFN who nearly all require phototherapy.

Intrauterine transfusions

Although IUTs are performed since the 1960s, there are still some aspects to be taken into consideration for future research in alloimmune HDFN. In our center, IUTs are performed mainly intravascular to treat fetal anemia. However, a combination of intravascular and intraperitoneal IUT might achieve a more stable fetal Ht and a longer interval between transfusions.[39-41] This topic deserves further study in a randomized controlled trial.
Another technical aspect of IUT that requires further investigation is the effect of analgesia on the fetal hormonal and hemodynamic stress response in case of intrahepatic vein needling.[42]
Further research is also required to evaluate the benefits and harms associated with maternal sedation and fetal paralysis during the procedures.[43]

Postnatal period

Exchange transfusions

Recently, an RCT that compared two-stage single-volume ET (TSSV-ET) with single-stage double-volume ET (SSDV-ET) in full-term neonates with HDFN due to Rh or ABO incompatibility showed promising results.[44] The mean rebound bilirubin level (measured 3 hours after ET) was significantly lower in the TSSV-ET group compared to the SSDV-ET group (12.7 ± 1.1 mg/dL versus 17.3 ± 1.7 mg/dL, p=<0.001). Also the need for repeated ET was significantly lower in the TSSV-ET group compared to the SSDV-ET group (13.5% versus 32.7%, p=<0.05). In addition, complications of ET (sepsis, NEC, pulmonary hemorrhage) and mortality were not significantly different between both groups. Authors suggested that the beneficial effect of TSSV-ET lies in lowering rebound bilirubin because extravascular bilirubin has more time to distribute to the intravascular space.[44]

Likewise, antibodies also have a longer period to distribute between the extravascular and intravascular space and consequently a lower antibody titer and duration and severity of late anemia could be expected. However, the authors did not measure the number of top-up transfusions or antibody titers.

Future RCTs should preferably include a more homogenous group of neonates (only Rh HDFN or ABO HDFN) and should also measure duration of phototherapy and the number op top-up transfusions to evaluate all possible benefits of TSSV-ET compared to SSDV-ET.

IVIg

Based on the results of the Cochrane review in Chapter 6 it can be concluded that the effectiveness of neonatal administration of IVIg in alloimmune HDN is still controversial. Future research into the role of neonatal administration of IVIg in alloimmune HDN may particularly be warranted in infants for whom ET is considered to be high risk or not available.

Prophylactic antibiotics

In Chapter 4 we demonstrated that leukocytopenia and sepsis are common complications of ET. Whether ET-related removal of leukocytes may play a role in the higher incidence of sepsis after ET deserves further study. However, it might be more interesting to evaluate the role of prophylactic antibiotics to prevent sepsis after ET in alloimmune HDFN because the higher incidence of sepsis is probably caused by manipulating the umbilical venous catheter during ET. A recent Cochrane review on the use of prophylactic antibiotics to reduce morbidity and mortality in neonates with umbilical venous catheters only included one

study which was of poor quality.[45] This quasi-RCT, performed in 1984, included 23 infants who required ET for hyperbilirubinemia and 6 infants who required partial ET for hematocrit > 0.70.[46] Although 5 of 15 infants given antibiotics and 5 of 14 control infants having positive blood cultures three days after catheterization, all positive blood cultures were considered contaminated due to lack of clinical and hematological evidence of infection.

Top-up transfusion and medicaments for late anemia

The optimal transfusion trigger for top-up transfusions in alloimmune HDN should be taken into consideration for future research. Guidelines for RBC transfusions in neonates vary greatly between and within countries. Possible advantages of restrictive transfusion guidelines are reduction of total transfusions and donor exposure. However, some studies reported worse short-term neurological or long-term neurodevelopmental outcome in those treated with restrictive transfusion guidelines compared to liberal transfusion guidelines.[47,48]

As previously mentioned in this chapter, RCTs to evaluate the use of rhEPO to prevent late anemia and consequently top-up transfusions are required. RhEPO has been increasingly used in neonates to prevent or reduce neonatal anemia without short or long-term adverse effects and might even work neuroprotective.[14,49-52]

Another benefit of rhEPO in alloimmune HDN could be lowering ferritin levels in neonates with iron overload/cholestasis. However, studies should monitor ferritin levels to prevent iron deficiency.

Folic acid and Vitamin E are often used in infants with alloimmune HDFN to support erythropoiesis.[53] However, no high-quality studies are performed so far to evaluate the efficacy of those supplements in reducing the severity of anemia. Further well-designed studies are required.

Long term outcome

There is lack of long-term follow-up studies in alloimmune HDFN. The neurodevelopmental outcome in IUT treated infants with alloimmune HDFN has recently been reported.[54] However, studies that evaluate long term effects on the immune system and liver function in infants with iron overload and/or cholestasis are warranted.

References

1. Diamond LK, Blackfan KD, Baty JM. Erythroblastosis fetalis and its association with universal edema of the fetus, icterus gravis neonatotum and anemia of the newborn. J.Pediatr. 1932;1:269-309.
2. Stockman JA, III. Overview of the state of the art of Rh disease: history, current clinical management, and recent progress. J.Pediatr.Hematol.Oncol. 2001 Aug;23(6):385-93.
3. Dorn I, Schlenke P, Hartel C. Prolonged anemia in an intrauterine-transfused neonate with Rh-hemolytic disease: no evidence for anti-D-related suppression of erythropoiesis in vitro. Transfusion 2010 May;50(5):1064-70.
4. Giblett ER, Varela JE, Finch CA. Damage of the bone marrow due to Rh antibody. Pediatrics 1956 Jan;17(1):37-44.
5. 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 Jul;165(1):79-81.
6. Koenig JM, Ashton RD, De Vore GR, Christensen RD. Late hyporegenerative anemia in Rh hemolytic disease. J.Pediatr. 1989 Aug;115(2):315-8.
7. Burk CD, Malatack JJ, Ramsey G. Misleading Rh phenotype and severe prolonged anemia in hemolytic disease of the newborn. Am.J.Dis.Child 1987 Jul;141(7):712-3.
8. 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 Aug;40(2):357-60.
9. 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 Jan;198(1):54.
10. Zuppa AA, Maragliano G, Scapillati ME, Florio MG, Girlando P, Noia G, De SM, Cavaliere AF, Romagnoli C, Tortorolo G. Recombinant erythropoietin in the prevention of late anaemia in intrauterine transfused neonates with Rh-haemolytic disease. Fetal Diagn.Ther. 1999 Sep;14(5):270-4.
11. Zuppa AA, Alighieri G, Calabrese V, Visintini F, Cota F, Carducci C, Antichi E, Noia GA, Fortunato G, Romagnoli C. Recombinant human erythropoietin in the prevention of late anemia in intrauterine transfused neonates with Rh-isoimmunization. J.Pediatr.Hematol.Oncol. 2010 Apr;32(3):e95-101.
12. 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 Aug;123(2):279-84.
13. 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 May;39(5):831-4.
14. Ohls RK, Wirkus PE, Christensen RD. Recombinant erythropoietin as treatment for the late hyporegenerative anemia of Rh hemolytic disease. Pediatrics 1992 Nov;90(5):678-80.
15. 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 Jan;17(1):22-4.
16. Pessler F, Hart D. Hyporegenerative anemia associated with Rh hemolytic disease: treatment failure of recombinant erythropoietin. J.Pediatr.Hematol.Oncol. 2002 Nov;24(8):689-93.

17. 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 Feb;192(2):478-82.
18. Voto LS, Mathet ER, Zapaterio JL, Orti J, Lede RL, Margulies M. High-dose gammaglobulin (IVIG) followed by intrauterine transfusions (IUTs): a new alternative for the treatment of severe fetal hemolytic disease. J.Perinat.Med. 1997;25(1):85-8.
19. Connan K, Kornman L, Savoia H, Palma-Dias R, Rowlands S. IVIG - is it the answer? Maternal administration of immunoglobulin for severe fetal red blood cell alloimmunisation during pregnancy: a case series. Aust.N.Z.J.Obstet.Gynaecol. 2009 Dec;49(6):612-8.
20. Fox C, Martin W, Somerset DA, Thompson PJ, Kilby MD. Early intraperitoneal transfusion and adjuvant maternal immunoglobulin therapy in the treatment of severe red cell alloimmunization prior to fetal intravascular transfusion. Fetal Diagn.Ther. 2008;23(2):159-63.
21. Ruma MS, Moise KJ, Jr., Kim E, Murtha AP, Prutsman WJ, Hassan SS, Lubarsky SL. Combined plasmapheresis and intravenous immune globulin for the treatment of severe maternal red cell alloimmunization. Am.J.Obstet.Gynecol. 2007 Feb;196(2):138-6.
22. Berlin G, Selbing A, Ryden G. Rhesus haemolytic disease treated with high-dose intravenous immunoglobulin. Lancet 1985 May 18;1(8438):1153.
23. De la Camara C, Arrieta R, Gonzalez A, Iglesias E, Omenaca F. High-dose intravenous immunoglobulin as the sole prenatal treatment for severe Rh immunization. N.Engl.J.Med. 1988 Feb 25;318(8):519-20.
24. Berlin G, Selbing A, Gottvall T. Plasma exchange and high-dose i.v. immunoglobulin in the treatment of severe rhesus hemolytic disease. Prog.Clin.Biol.Res. 1990;337:337-40.
25. Margulies M, Voto LS, Mathet E, Margulies M. High-dose intravenous IgG for the treatment of severe rhesus alloimmunization. Vox Sang. 1991;61(3):181-9.
26. Fernandez-Jimenez MC, Jimenez-Marco MT, Hernandez D, Gonzalez A, Omenaca F, de la Camara C. Treatment with plasmapheresis and intravenous immunoglobulin in pregnancies complicated with anti-PP1Pk or anti-K immunization: a report of two patients. Vox Sang. 2001 Feb;80(2):117-20.
27. Gottvall T, Selbing A. Alloimmunization during pregnancy treated with high dose intravenous immunoglobulin. Effects on fetal hemoglobin concentration and anti-D concentrations in the mother and fetus. Acta Obstet.Gynecol.Scand. 1995 Nov;74(10):777-83.
28. Scott JR, Branch DW, Kochenour NK, Ward K. Intravenous immunoglobulin treatment of pregnant patients with recurrent pregnancy loss caused by antiphospholipid antibodies and Rh immunization. Am.J.Obstet.Gynecol. 1988 Nov;159(5):1055-6.
29. Deka D, Buckshee K, Kinra G. Intravenous immunoglobulin as primary therapy or adjuvant therapy to intrauterine fetal blood transfusion: a new approach in the management of severe Rh-immunization. J.Obstet.Gynaecol.Res. 1996 Dec;22(6):561-7.
30. Porter TF, Silver RM, Jackson GM, Branch DW, Scott JR. Intravenous immune globulin in the management of severe Rh D hemolytic disease. Obstet.Gynecol.Surv. 1997 Mar;52(3):193-7.
31. Chitkara U, Bussel J, Alvarez M, Lynch L, Meisel RL, Berkowitz RL. High-dose intravenous gamma globulin: does it have a role in the treatment of severe erythroblastosis fetalis? Obstet.Gynecol. 1990 Oct;76(4):703-8.
32. Alonso JG, Decaro J, Marrero A, Lavalle E, Martell M, Cuadro JC. Repeated direct fetal intravascular high-dose immunoglobulin therapy for the treatment of Rh hemolytic disease. J.Perinat.Med. 1994;22(5):415-9.

33. Dooren MC, Van Kamp IL, Scherpenisse JW, Brand R, Ouwehand WH, Kanhai HH, Engelfriet CP, Gravenhorst JB. No beneficial effect of low-dose fetal intravenous gammaglobulin administration in combination with intravascular transfusions in severe Rh D haemolytic disease. Vox Sang. 1994;66(4):253-7.
34. Kriplani A, Malhotra SB, Mandal K. Fetal intravenous immunoglobulin therapy in rhesus hemolytic disease. Gynecol.Obstet.Invest 2007;63(3):176-80.
35. Teune MJ, Bakhuizen S, Gyamfi BC, Opmeer BC, van Kaam AH, van Wassenaer AG, Morris JM, Mol BW. A systematic review of severe morbidity in infants born late preterm. Am.J.Obstet.Gynecol. 2011 Oct;205(4):374-9.
36. Gyamfi-Bannerman C. The scope of the problem: the epidemiology of late preterm and early-term birth. Semin.Perinatol. 2011 Oct;35(5):246-8.
37. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane.Database.Syst.Rev. 2006;(3):CD004454.
38. 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.
39. Nicolini U, Kochenour NK, Greco P, Letsky E, Rodeck CH. When to perform the next intra-uterine transfusion in patients with Rh allo-immunization: combined intravascular and intraperitoneal transfusion allows longer intervals. Fetal Ther. 1989;4(1):14-20.
40. Santolaya J, Warsof SL. Combined intravascular-intraperitoneal transfusions in hydropic twins due to Rh (D) alloimmunization. Fetal Diagn.Ther. 1990;5(2):70-5.
41. Moise KJ, Jr., Carpenter RJ, Jr., Kirshon B, Deter RL, Sala JD, Cano LE. Comparison of four types of intrauterine transfusion: effect on fetal hematocrit. Fetal Ther. 1989;4(2-3):126-37.
42. Fisk NM, Gitau R, Teixeira JM, Giannakoulopoulos X, Cameron AD, Glover VA. Effect of direct fetal opioid analgesia on fetal hormonal and hemodynamic stress response to intrauterine needling. Anesthesiology 2001 Oct;95(4):828-35.
43. Dodd JM, Windrim RC, Van Kamp IL. Techniques of intrauterine fetal transfusion for women with red-cell isoimmunisation for improving health outcomes. Cochrane.Database.Syst.Rev. 2012;9:CD007096.
44. 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 Jul;25(7):1080-3.
45. Inglis GD, Davies MW. Prophylactic antibiotics to reduce morbidity and mortality in neonates with umbilical venous catheters. Cochrane.Database.Syst.Rev. 2005;(4):CD005251.
46. Pulido N, Montesinos A, Arriaza M, Esparza P. [Prophylactic use of antibiotics in umbilical catheterization in newborn infants]. Rev.Chil.Pediatr. 1985 Jul;56(4):247-9.
47. Bell EF, Strauss RG, Widness JA, Mahoney LT, Mock DM, Seward VJ, Cress GA, Johnson KJ, Kromer IJ, Zimmerman MB. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. Pediatrics 2005 Jun;115(6):1685-91.
48. Whyte RK, Kirpalani H, Asztalos EV, Andersen C, Blajchman M, Heddle N, LaCorte M, Robertson CM, Clarke MC, Vincer MJ, et al. Neurodevelopmental outcome of extremely low birth weight infants randomly assigned to restrictive or liberal hemoglobin thresholds for blood transfusion. Pediatrics 2009 Jan;123(1):207-13.
49. Ohls RK, Ehrenkranz RA, Das A, Dusick AM, Yolton K, Romano E, Delaney-Black V, Papile LA, Simon NP, Steichen JJ, et al. Neurodevelopmental outcome and growth at 18 to 22 months' corrected age in extremely low birth weight infants treated with early erythropoietin and iron. Pediatrics 2004 Nov;114(5):1287-91.

50. 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;3:CD004865.
51. 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.
52. Juul S. Neuroprotective role of erythropoietin in neonates. J.Matern.Fetal Neonatal Med. 2012 Oct;25 Suppl 4:105-7.
53. Rath ME, Smits-Wintjens VE, Walther FJ, Lopriore E. Hematological morbidity and management in neonates with hemolytic disease due to red cell alloimmunization. Early Hum.Dev. 2011 Sep;87(9):583-8.
54. Lindenburg IT, Smits-Wintjens VE, van Klink JM, Verduin E, Van Kamp IL, Walther FJ, Schonewille H, Doxiadis II, Kanhai HH, van Lith JM, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am.J.Obstet.Gynecol. 2012 Feb;206(2):141-8.

Nederlandse samenvatting

Hemolytische ziekte van de foetus en de pasgeborene (HZFP) is een aandoening waarbij rode bloedcellen van de foetus/pasgeborene worden afgebroken door antistoffen (afweerstoffen) die van de moeder afkomstig zijn.
Op de rode bloedcellen zitten eiwitten (bloedgroepantigenen) die de bloedgroep bepalen. Deze bloedgroepantigenen zijn niet bij alle mensen hetzelfde. Een voorbeeld van een dergelijk bloedgroepantigeen is rhesus-D. Als de rode bloedcellen van een moeder dit bloedgroepantigeen niet bevatten (rhesus-D-negatief) en die van haar foetus wel (rhesus-D-positief), dan kan de moeder antistoffen (rhesus-D-antistoffen) gaan maken tegen de bloedcellen van haar kind. Dit proces, dat zwangerschapsimmunisatie genoemd wordt, kan ontstaan als bloedcellen van de foetus in de bloedbaan van de moeder terecht komen. Dit gebeurt meestal tijdens de bevalling, maar kan ook in de laatste drie maanden van de zwangerschap ontstaan. Als tijdens een zwangerschap of de bevalling rhesus-D-antistoffen zijn gevormd, is het afweersysteem van de moeder geactiveerd. Bij een volgende zwangerschap van een rhesus-D-positief kind reageert het afweersysteem van de moeder snel met het aanmaken van rhesus-D-antistoffen als bloed van de foetus in de bloedbaan van de moeder terecht komt. Deze antistoffen kunnen via de placenta bij de foetus terecht komen en daar voor afbraak van de rode bloedcellen (hemolyse) van de foetus zorgen. Dit kan leiden tot bloedarmoede (anemie) bij de foetus. Soms is de bloedarmoede zo ernstig dat één of meerdere zogenaamde intra-uteriene bloedtransfusies (IUTs) nodig zijn. Deze IUTs worden in de foetus zelf toegediend via de navelstreng of een lever vene.
Ook na de geboorte kan anemie een probleem zijn en daarnaast kan het kind geel gaan zien (icterus) en ziek worden van de ophoping van bilirubine, een afbraakproduct van de rode bloedcellen, in het bloed en in de weefsels. Het teveel aan bilirubine in het bloed (hyperbilirubinemie) moet behandeld worden omdat het schadelijk kan zijn voor de hersenen. Bilirubine wordt in de lever omgezet in een wateroplosbare vorm die via de gal en urine kan worden uitgescheden. Deze omzetting wordt conjugatie genoemd. Bij ernstige hyperbilirubinemie kan de lever niet al het bilirubine omzetten en kan het ongeconjugeerde bilirubine, dat de bloed-hersen-barrière kan passeren, zich afzetten in het hersenweefsel en tot ernstige hersenschade (kernicterus) met blijvende neurologische verschijnselen en gehoorverlies leiden en zelfs tot overlijden.
De behandeling van hyperbilirubinemie begint met intensieve fototherapie, waarbij het ongeconjugeerde bilirubine onder invloed van licht wordt omgezet in een wateroplosbare vorm. Als het bilirubine in het bloed ondanks de fototherapie verder blijft stijgen, kan een wisseltransfusie noodzakelijk zijn. Bij een wisseltransfusie wordt het bloed van het kind vervangen door donorbloed door steeds kleine hoeveelheden bloed van het kind af te nemen en hier donorbloed voor terug te geven via een katheter in de navel. In totaal wordt

meestal tweemaal het circulerend bloedvolume van de pasgeborene gewisseld (160 ml/kg). Met een wisseltransfusie worden bilirubine en antistoffen verwijderd en wordt eventuele anemie behandeld.
De antistoffen kunnen na de geboorte nog een aantal maanden in het bloed van het kind aanwezig zijn en voor afbraak van rode bloedcellen zorgen. Rode bloedceltransfusies (top-up transfusies) bij kinderen met HZFP kunnen tot ongeveer drie maanden na de geboorte noodzakelijk zijn.
Naast bloedarmoede kunnen de volgende hematologische problemen voorkomen bij HZFP: te laag aantal bloedplaatjes (trombocytopenie) en witte bloedcellen (leukocytopenie), stollingsstoornissen en ijzerstapeling.

In **Hoofdstuk 2** wordt een samenvatting van de literatuur gegeven van de hematologische problemen die bij HZFP kunnen voorkomen. Tevens worden de behandelingsopties van deze problemen besproken.

Deel 1 Wisseltransfusies
In **Hoofdstuk 3** beschreven wij het effect van de verandering in onze richtlijn fototherapie en wisseltransfusies op de bloedtransfusiebehoefte van kinderen met HZFP. In december 2005 zijn naar aanleiding van aanbevelingen van de American Academy of Pediatrics de wisseltransfusie-criteria in ons centrum aangepast. Door deze aanpassing hoefde veel minder vaak een wisseltransfusie uitgevoerd te worden. Voor deze studie hebben wij het aantal wisseltransfusies en top-up transfusies vergeleken tussen een groep (bijna) voldragen pasgeborenen met HZFP die volgens de oude richtlijn werden behandeld (groep 1, n=156) met pasgeborenen met HZFP die volgens de nieuwe richtlijn werden behandeld (groep 2, n=27). Het percentage pasgeborenen bij wie een wisseltransfusie werd uitgevoerd daalde van 66% (103/156) in groep 1 naar 26% (7/27) in groep 2. Het percentage pasgeborenen aan wie een top-up transfusie werd gegeven steeg echter van 68% (105/154) in groep 1 naar 81% (22/27) in groep 2. Het aantal top-up transfusies was significant hoger in groep 2 dan in groep 1. Wij hebben hiermee aangetoond dat de nieuwe richtlijn heeft geleid tot sterke vermindering van het aantal wisseltransfusies, maar ook een toename van het aantal top-up transfusies.

In **Hoofdstuk 4** rapporteerden wij de resultaten van een studie naar de complicaties van wisseltransfusies bij pasgeborenen met HZFP. Een wisseltransfusie is een risicovolle, invasieve procedure waarbij katheters in de bloedbaan (meestal in de navelstrengader) worden gebracht. Bijwerkingen die beschreven zijn omvatten onder andere: infecties,

elektrolytstoornissen, hematologische complicaties en cardiorespiratoire complicaties. Wij hebben bij in totaal 347 pasgeborenen met HZFP gekeken naar het aantal complicaties ten gevolge van wisseltransfusies. Daartoe hebben wij alle complicaties geregistreerd van alle pasgeborenen met HZFP die ten minste één wisseltransfusie hebben ondergaan (ET-groep, n=134) en vergeleken met degenen die geen wisseltransfusie hebben ondergaan (niet-ET-groep, n=213). Vergelijking tussen ET-groep en niet-ET-groep liet zien dat behandeling met wisseltransfusie onafhankelijk geassocieerd is met: klinisch beeld van sepsis (bloedvergiftiging) met positieve bloedkweek (8% in ET-groep versus 1% in niet-ET-groep), leukocytopenie (88% versus 23%), ernstige trombocytopenie (bloedplaatjes <50 x 10^9/L) (63% versus 8%), hypocalciëmie (te laag calciumgehalte in het bloed) (22% versus 1%) en hypernatriëmie (te hoog natriumgehalte in het bloed) (8% versus 0%). Deze complicaties waren allemaal van voorbijgaande aard en neonatale sterfte kwam niet voor in de ET-groep. Uit deze studie kunnen wij dus opmaken dat permanente ziekte en sterfte ten gevolge van wisseltransfusies bij pasgeborenen met HZFP in ervaren handen tot een minimum beperkt kan worden.

Deel 2 Intraveneus immunoglobulinen

Omdat een wisseltransfusie een invasieve, risicovolle procedure is, wordt naar alternatieve behandelingen voor hyperbilirubinemie gezocht. Eén van deze alternatieven is intraveneus immunoglobulinen (IVIg). In de Amerikaanse richtlijn uit 2004 wordt geadviseerd om IVIg toe te dienen aan pasgeborenen met HZFP als fototherapie niet voldoende is om de hyperbilirubinemie te behandelen. Deze richtlijn was echter gebaseerd op kleine studies met kwalitatieve beperkingen. De Cochrane review uit 2002 die het effect van IVIg op het aantal wisseltransfusies onderzocht, concludeerde dat meer studies (van hoge kwaliteit) noodzakelijk waren om een uitspraak over het effect van IVIg te kunnen doen.

In **Hoofdstuk 5** hebben wij in het Leids Universitair Medisch Centrum met een gerandomiseerde dubbelblinde placebo-gecontroleerde studie (LIVIN-studie) onderzocht of het vlak na de geboorte geven van IVIg (0.75 g/kg) het aantal wisseltransfusies kan verminderen bij pasgeborenen met HZFP door rhesus-D- of rhesus-c-antistoffen. In totaal kregen 41 pasgeborenen IVIg en 39 pasgeborenen een placebo (glucose 5% oplossing via het infuus). Het percentage pasgeborenen dat ten minste één wisseltransfusie nodig had, was niet verschillend tussen de IVIg-groep en placebogroep (17% versus 15% respectievelijk). Ook de duur van de fototherapie (4.7 versus 5.1 dagen), de gemiddelde maximale bilirubine waarde tijdens opname (14.8 versus 14.1 mg/dL) en het percentage pasgeborenen dat ten minste één top-up transfusie in de eerste drie maanden na de

geboorte nodig had (83% versus 87%) was niet verschillend tussen beide groepen. Deze bevindingen ondersteunen het standaard gebruik van IVIg bij het falen van fototherapie niet.

In **Hoofdstuk 6** beschreven wij de resultaten van de update van de Cochrane review uit 2002 die het effect van IVIg op het aantal en de noodzaak voor wisseltransfusies onderzocht bij pasgeborenen met HZFP. Voor dit onderzoek (meta-analyse) hebben we de resultaten van veertien (quasi-) gerandomiseerde studies met een controlegroep samengevoegd om een betrouwbaardere uitspraak te kunnen doen over het effect van IVIg. Slechts twee van de veertien studies (waaronder de LIVIN-studie) waren van hoge kwaliteit. Meta-analyse van alle veertien onderzoeken, met in totaal 942 kinderen, toonde een vermindering in het aantal en de noodzaak voor wisseltransfusies bij pasgeborenen die met IVIg en fototherapie behandeld werden vergeleken met pasgeborenen die met alleen fototherapie (en eventuele placebo) werden behandeld. Echter, analyse van de twee studies van hoge kwaliteit toonde geen vermindering van het aantal en de noodzaak voor wisseltransfusies wanneer IVIg werd gecombineerd met intensieve fototherapie. In acht studies werd het effect van IVIg op de top-up transfusiebehoefte voor late bloedarmoede onderzocht. Meta-analyse van deze studies toonde geen verschil in het aantal en de noodzaak voor top-up transfusies tussen pasgeborenen die met en zonder IVIg werden behandeld. Op basis van het bewijs van de twee studies van hoge kwaliteit kan geconcludeerd worden dat IVIg niet effectief is in het voorkomen van wisseltransfusies of top-up transfusies en moet routinematig gebruik van IVIg bij pasgeborenen met HZFP worden afgeraden. Aangezien er wel aanwijzingen zijn (in laboratoriumstudies) dat IVIg hemolyse vermindert, zijn toekomstige hoge kwaliteit studies nodig om te bepalen of IVIg een beperkte rol heeft bij een selecte groep pasgeborenen met HZFP, bijvoorbeeld wanneer een wisseltransfusie niet tijdig of zonder hoog risico op complicaties kan worden uitgevoerd.

Deel 3 Alloimmunisaties anders dan rhesus-D

Anti-Kell en anti-c zijn na anti-D de belangrijkste antistoffen die ernstige HZFP veroorzaken waarvoor IUT vaak noodzakelijk is. Anti-Kell is, na anti-D, ook de meest voorkomende antistof die HZFP in Nederland veroorzaakt. Kell HZFP heeft waarschijnlijk een andere pathogenese (ontstaanswijze van ziekte) dan rhesus-D HZFP. Kell HZFP lijkt meer een probleem van de aanmaak van rode bloedcellen dan van afbraak (hemolyse) van rode bloedcellen. Net zoals bij rhesus-D HZFP kunnen bij pasgeborenen met Kell HZFP bloedtransfusies noodzakelijk zijn tot enkele maanden na de geboorte.

In **Hoofdstuk 7** hebben we bekeken of de incidentie en ernst van anemie verschilt tussen pasgeborenen met rhesus-D en Kell HZFP. Wij vonden dat pasgeborenen met Kell HZFP minder fototherapie (2.4 versus 4.1 dagen) en wisseltransfusies (6% versus 62%) nodig hadden dan pasgeborenen met rhesus-D HZFP. De noodzaak van en het aantal top-up transfusies en het aantal dagen na de geboorte tot de eerste top-up transfusie waren niet significant verschillend tussen beide groepen. Onze resultaten laten zien dat kinderen met Kell HDFN een ander beleid vlak na de geboorte vereisen (minder fototherapie en minder wisseltransfusies), maar hetzelfde follow-up beleid als kinderen met rhesus-D HZFP.

In **Hoofdstuk 8** hebben we in een vergelijkbare studie als Hoofdstuk 7 de uitkomsten vergeleken tussen kinderen met rhesus-c HZFP en rhesus-D HZFP. We vonden dat kinderen met rhesus-c HZFP gemiddeld eerder en vaker een IUT nodig hadden dan kinderen met rhesus-D HZFP (61% versus 41%). Het beloop na de geboorte was niet significant verschillend tussen de kinderen met rhesus-c en rhesus-D HZFP in termen van duur van fototherapie (gemiddeld 4.8 en 4.5 dagen), noodzaak voor een wisseltransfusie (50% versus 46%) en de noodzaak van top-up transfusies (62% versus 78%). Daarom is ook voor rhesus-c HZFP hetzelfde follow-up beleid als rhesus-D HZFP gerechtvaardigd. In deze studie hebben we ook onderzocht of de antistoftiter bij de geboorte gecorreleerd is met de transfusiebehoeften na de geboorte. We vonden een positieve correlatie tussen anti-D-titer bij de geboorte en de noodzaak van en het aantal top-up transfusies. Daarnaast vonden we een positieve correlatie tussen anti-c-titer bij de geboorte en de noodzaak voor wisseltransfusie.

Deel 4 Geassocieerde hematologische problemen

In **Hoofdstuk 9** hebben we gekeken naar de incidentie en ernst van trombocytopenie bij de geboorte en de risicofactoren voor het krijgen hiervan onderzocht bij pasgeborenen met HZFP. Wij vonden dat 26% (94/362) trombocytopenie (bloedplaatjesgehalte <150 x 10^9/L) en 6% (20/362) ernstige trombocytopenie (bloedplaatjesgehalte <50 x 10^9/L) bij de geboorte had. Eén pasgeborene met trombocytopenie had klinische symptomen van een bloeding (intraventriculaire bloeding graad 2) bij de geboorte. Zeer waarschijnlijk hebben de prematuriteit (te vroeg geboren zijn) van deze pasgeborene en hydrops (vochtophoping in organen/huid bij ernstige HZFP) ook meegespeeld in het ontwikkelen van de bloeding in de hersenen. We hebben drie risicofactoren voor het ontstaan van trombocytopenie bij de geboorte bij kinderen met HZFP gevonden: behandeling met IUT, dysmaturiteit (te laag geboortegewicht voor de zwangerschapsduur) en prematuriteit.

In **Hoofdstuk 10** hebben we de incidentie, potentiële risicofactoren, het behandelbeleid en de uitkomsten van cholestase (verstoring van de galafvoer) bij pasgeborenen met HZFP bekeken. Cholestase is een ziekte van de lever waarbij het geconjugeerde bilirubine in het bloed te hoog is. De oorzaak van cholestase bij HZFP is niet precies bekend, maar lijkt samen te hangen met ijzerstapeling in de lever ten gevolge van (intra-uteriene) rode bloedceltransfusies. Cholestase kwam voor bij 13% (41/313) en was onafhankelijk geassocieerd met IUT behandeling en rhesus-D type immunisatie. Echter, 88% van de pasgeborenen met cholestase had zowel rhesus-D type immunisatie en werd behandeld met IUT, wat een betrouwbaar onderscheid tussen beide risicofactoren niet mogelijk maakt. Uitgebreide tests om andere oorzaken van cholestase uit te sluiten waren allen negatief (oorzaak niet aangetoond). Echter, ze werden niet bij alle pasgeborenen met cholestase uitgevoerd. In bijna de helft van de patiënten met cholestase herstelde dit spontaan binnen 1 week tot 3 maanden na de geboorte. In de andere helft van de patiënten werden bilirubine en leverenzymen in het bloed niet gecontroleerd tot zij normale waarden hadden bereikt. Eén patiënt met cholestase en ernstige ijzerstapeling werd behandeld met ontijzeringstherapie. Grotere vervolgstudies zijn nodig om het beloop en de oorzaak van cholestase bij kinderen met HZFP vast te stellen.

In **Hoofdstuk 11** hebben wij het beloop van de ijzerstatus in de eerste drie levensmaanden in het bloed bij kinderen met HZFP bestudeerd. We vonden ijzerstapeling (gedefinieerd als te hoog ferritine gehalte in het bloed voor de leeftijd) bij 70% (23/33) van de pasgeborenen bij de geboorte, bij 50% (10/20) op de leeftijd van één maand, bij 42% (5/12) op de leeftijd van twee maanden en bij 18% (2/11) op de leeftijd van drie maanden. IJzerdeficiëntie kwam niet voor in ons cohort, behalve bij één voldragen pasgeborene op de leeftijd van drie maanden op basis van het voor dit onderzoek bepaald criterium voor ijzerdeficiëntie (ferritine gehalte bloed <40 ug/L). Of dit kind, dat één IUT en geen postnatale transfusies heeft gehad, baat zou hebben gehad bij ijzersuppletie is niet duidelijk. In ons cohort kregen geen van de kinderen ijzersuppletie. Wij raden aan de ijzerstatus te meten voor het starten van ijzersuppletie bij kinderen met HZFP, omdat zowel ijzergebrek als ijzerstapeling nadelige effecten kunnen hebben. Om de lange termijn uitkomsten van ijzerstapeling en ijzerdeficiëntie bij kinderen met HZFP in kaart te brengen, is meer onderzoek nodig.

Conclusie

Dit proefschrift richt zich op een aantal aspecten van de hematologische uitkomst van zuigelingen met HZFP als gevolg van zwangerschapsimmunisatie, inclusief de ontstaanswijze en het behandelbeleid van de ziekte. De aanwezigheid van trombocytopenie en

leukocytopenie ondersteunen het mechanisme van onderdrukking van de aanmaak van bloedplaatjes en witte bloedcellen door een verhoogde aanmaak van rode bloedcellen. Naast de problemen veroorzaakt door een tekort aan bloedplaatjes en witte en rode bloedcellen, draagt de overmaat van bilirubine en ijzer (metabolieten van rode bloedcellen) ook bij aan de morbiditeit van de ziekte. Op basis van onze systematische review kunnen we ook concluderen dat er onvoldoende bewijs is voor het gebruik van IVIg bij deze ziekte. Ongeacht de controverse over het gebruik van IVIg, blijkt uit ons onderzoek dat intensieve follow-up is aangewezen in zowel rhesus-D als niet-rhesus-D HZFP en bewustwording over de daarmee gepaard gaande morbiditeit van groot belang is.

Authors and affiliations

Publications

Curriculum Vitae

Dankwoord

Abbreviations and acronyms

Authors and affiliations

From the Division of Neonatology, Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands:
Enrico Lopriore, Arjan B. te Pas, Mirjam E.A. Rath, Vivianne E.H.J. Smits-Wintjens, Sylke J. Steggerda, Frans J. Walther

From the Division of Fetal Medicine, Department of Obstetrics, Leiden University Medical Center, Leiden, the Netherlands:
Irene T.M. Lindenburg, Inge L. van Kamp, Dick Oepkes

From the Department of Immuno-Hematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands:
Anneke Brand

From the Department of Biostatistics, Leiden University Medical Center, Leiden, the Netherlands:
Erik W. van Zwet

From Sanquin Blood Supply Foundation, Amsterdam, the Netherlands:
Christine M. Kramer, Claudia C. Folman

From the Division of Neonatology, Department of Pediatrics, Mater Mothers' Hospital, South Brisbane, Australia:
Helen Liley

Publications

1. *Rath ME*, Smits-Wintjens VE, Lopriore E, Liley H. Immunoglobulin for alloimmune hemolytic disease in neonates. Cochrane Database Syst Rev; Invited

2. *Rath ME*, Smits-Wintjens VE, Lindenburg IT, Folman CC, Brand A, van Kamp IL, Oepkes D, Walther FJ, Lopriore E. Postnatal outcome in neonates with severe Rhesus c compared to Rhesus D hemolytic disease. Transfusion 2012; Epub ahead of print

3. *Rath ME* & Smits-Wintjens VE, van Zwet EW, Oepkes D, Brand A, Walther FJ, Lopriore E. Neonatal morbidity after exchange transfusion for red cell alloimmune hemolytic disease. Neonatology 2012; 103(2):141-147

4. Spruijt M, Steggerda S, *Rath M*, van Zwet E, Oepkes D, Walther F, Lopriore E. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstet Gynecol. 2012 Jul; 120(1):15-20

5. Smits-Wintjens VE, *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(4):306-10

6. *Rath ME* & Smits-Wintjens VE, Oepkes D, van Zwet EW, van Kamp IL, Brand A, Walther FJ, Lopriore E. Thrombocytopenia at birth in neonates with red cell alloimmune haemolytic disease. Vox Sang. 2012 Apr; 102(3):228-33

7. *Rath ME*, Smits-Wintjens VE, Walther FJ, Lopriore E. Hematological morbidity and management in neonates with hemolytic disease due to red cell alloimmunization. Early Hum Dev. 2011 Sep; 87(9):583-8

8. Smits-Wintjens VE, Walther FJ, *Rath ME*, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D, Brand A, Lopriore E. Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. Pediatrics. 2011 Apr; 127(4):680-6

9. *Rath ME*, Smits-Wintjens VE, Lindenburg IT, Brand A, van Kamp IL, Oepkes D, Walther FJ, Lopriore E. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sang. 2011 Apr;100(3):312-6

10. *Rath ME*, Smits-Wintjens VE, 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 Jul 1; 99(1):65-70

Curriculum Vitae

Mirjam Rath werd geboren op 24 november 1983 te ’s-Gravenhage. In 2002 behaalde zij haar Gymnasium diploma aan de Dalton Scholengemeenschap locatie Aronskelkweg te ’s-Gravenhage. In hetzelfde jaar begon zij aan haar studie Geneeskunde aan de Universiteit Leiden. Zij onderbrak haar studie van 2005-2006 om zich een jaar lang in te zetten als bestuurslid van de Medische Faculteit der Leidse Studenten (M.F.L.S.). In 2009 studeerde zij cum laude af voor Geneeskunde. Haar wetenschappelijke stage voor Geneeskunde heeft zij op de afdeling Neonatologie van het LUMC uitgevoerd. Na het behalen van een twee-jarige MD-PhD-beurs binnen het LUMC, heeft zij deze wetenschapsstage tot een promotieonderzoek, onder begeleiding van prof. dr. F.J. Walther en dr. E. Lopriore, uit kunnen breiden. Dit onderzoek, dat zij in 2012 heeft afgerond, heeft geleid tot dit proefschrift. In de periode tussen haar wetenschapsstage en het begin van haar promotieonderzoek heeft zij ruim een jaar als arts-assistent niet in opleiding gewerkt in het HagaZiekenhuis locatie Juliana Kinderziekenhuis te Den Haag. Per 1 januari 2013 is zij begonnen met de opleiding tot Kinderarts in het LUMC (opleider: prof. dr. F.J. Walther).

Dankwoord

Dit proefschrift had ik niet in de relatief korte periode kunnen vervaardigen zonder goede begeleiding en hulp van anderen. Hierbij wil ik iedereen die mij heeft geholpen bedanken en in het bijzonder de volgende drie personen.

Allereerst mijn promotor, prof. dr. Frans J. Walther.
Beste professor Walther, dank dat u mij tijdens het keuzevak Neonatologie enthousiast hebt gemaakt en mij de mogelijkheid hebt gegeven om mijn wetenschapsstage en promotieonderzoek op de afdeling Neonatologie te komen verrichten. Als promotor was u altijd betrokken en hield u de grote lijnen in de gaten. Bedankt voor het vertrouwen en uw adviezen op wetenschappelijk en carrière gebied!

Mijn co-promotor, dr. Enrico Lopriore.
Enrico, jouw enthousiasme werkt aanstekelijk! Tijdens mijn wetenschapsstage had je steeds weer nieuwe ideeën, corrigeerde mijn werk binnen no-time en was daarbij ook lekker kritisch. Jouw voorstel om die wetenschapsstage uit te breiden tot een promotie zag ik dan ook gelijk helemaal zitten. Toen we uiteindelijk die beurs hadden binnengehaald, heb je me gelukkig weten te overtuigen de sollicitatiegesprekken voor de opleiding tot kinderarts af te zeggen. Grazie mille voor jouw overtuigingskracht, begeleiding, ideeën, adviezen, geduld en humor!

Mijn grote voorbeeld dr. Vivianne E.H.J. Smits-Wintjens.
Lieve Vivianne, bewondering heb ik voor jou: promoveren naast je werk als neonatoloog en moeder van drie kinderen zijn, mèh mie lêpke! (hoe is het mogelijk!) Samenwerken met jou is fantastisch. En om met jouw eigen woorden te spreken: dat we nog héél lang samen artikelen mogen schrijven! Bedankt voor alles!

Daarnaast wil ik alle co-auteurs bedanken voor hun waardevolle commentaren: prof. dr. Anneke Brand, dr. Inge van Kamp, prof. dr. Dick Oepkes, Irene Lindenburg, dr. Erik van Zwet, Christine Kramer, dr. Arjan te Pas en dr. Claudia Folman, dank jullie wel! And last but not least, dr. Helen Liley, dear Helen, thank you for all your advices and it was a great pleasure to work with you!

Al mijn collega-onderzoekers en kamergenoten wil ik bedanken voor de gezellige en leerzame tijd samen. In het bijzonder bedank ik: Esther Rijntjes, Jeroen van Vonderen, dr.

Jeannette von Lindern, dr. Sandra Prins, Jeanine van Klink, dr. Kim Schilleman, Marita de Waard, Margot Visser, Liset Elsgeest, Corinne van der Pot en Nadia Narayen.

Alle neonatologen, fellows, arts-assistenten, physician assistants en verpleegkundigen van de afdeling Neonatologie van het LUMC wil ik ook bedanken voor de leerzame en gezellige momenten tijdens mijn onderzoeksperiode.

Voor de ondersteunende werkzaamheden wil ik alle secretaresses van het stafsecretariaat en de afdeling Neonatologie bedanken, in het bijzonder: Wendy Matthijsen, Mirjam Vollebregt, Karin Ooijendijk, Francis Pauwels en Els Straathof.

Mijn vrienden en vriendinnen, in het bijzonder de Clovers en de Wateringse meiden, wil ik bedanken voor hun steun en interesse in mijn onderzoek. In het bijzonder bedank ik jou, Debbie Horsten, lieve Buufster, voor het samen lachen, huilen, spuien, creatief denken en al het andere dat jou zo'n goede vriendin maakt. Fijn dat je me ook als paranimf bijstaat!

Tot slot wil ik mijn familie bedanken voor hun vertrouwen, interesse en steun tijdens deze waardevolle tijd als onderzoekster en de tijd die daar vooraf aan is gegaan. Mijn moeder, Francis Boesaard, lieve mam, bedankt voor de liefdevolle opvoeding, je kritische kijk op dingen, het altijd meedenken, je liefde en al het andere dat je me hebt gegeven. Mijn vader, Hiltjo Rath, lieve pap, bedankt voor je relativerende woorden, je trots, waardering en liefde en al het andere dat je me hebt gegeven. Mijn zus, Judith van Veldhoven-Rath, lieve Juud, dank je wel dat je als kleine grote zus er altijd voor mij bent! Wat geweldig dat jij, samen met mijn toekomstig neefje of nichtje, als paranimf achter mij staat! De vriendin van mijn vader, Cil Hoogendijk, lieve Cil, ook jou wil ik bedanken voor je steun en interesse en je kookkunsten niet te vergeten.
Maar de beste kok is Wouter Scheffer. Lieve Wouter, ook al is de liefde tussen ons nog pril, het voelt heel goed met jou! Bedankt voor je geduld en de steun, afleiding, rust en liefde die je me tijdens de afronding van dit boekje hebt gegeven!

Abbreviations and acronyms

AAP	American Academy of Pediatrics
ADCC	Antibody Dependent Cellular Cytotoxicity
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CI	Confidence interval
EPO	Erythropoietin
ET(s)	Exchange transfusions
FNAIT	Fetal/neonatal alloimmune thrombocytopenia
γGT	Gamma-glutamyl transpeptidase
Hb	Hemoglobin
HELLP	(syndrome of) Hemolysis, Elevated Liver enzymes, Low Platelet counts
HDFN	Hemolytic disease of the fetus/newborn
HDN	Hemolytic disease of the newborn/neonate
Ht	Hematocrit
IgG	Immunoglobulin G
IQR	Interquartile range
ITP	Immune thrombocytopenic purpura
IUT(s)	Intrauterine transfusion(s)
IVIg	Intravenous immunoglobulin
LDH	Lactate dehydrogenase
LIVIN	Leiden's IVIg trial in Rhesus disease of the Neonate
LUMC	Leiden University Medical Center
MCA	Middle cerebral artery
MD	Mean difference
MRI	Magnetic resonance imaging
NICU	Neonatal intensive care unit
NEC	Necrotizing enterocolitis
NNT	Number needed to treat
OR	Odds ratio
PIH	Pregnancy induced hypertension
PVT	Portal vein thrombosis
RBC	Red blood cell
RCT	Randomized controlled trial
Rh	Rhesus

rhEPO	Recombinant human erythropoietin
RR	Relative risk/risk ratio
SD	Standard deviation
SGA	Small for gestational age
TORCH	Toxoplasma gondii, Rubella virus, Cytomegalovirus, Herpes Simplex virus
TIBC	Total iron binding capacity
TSB	Total serum bilirubin
UVC	Umbilical venous catheter