Neonatal management and outcome in red cell alloimmunization

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Vivianne Smits - Wintjens

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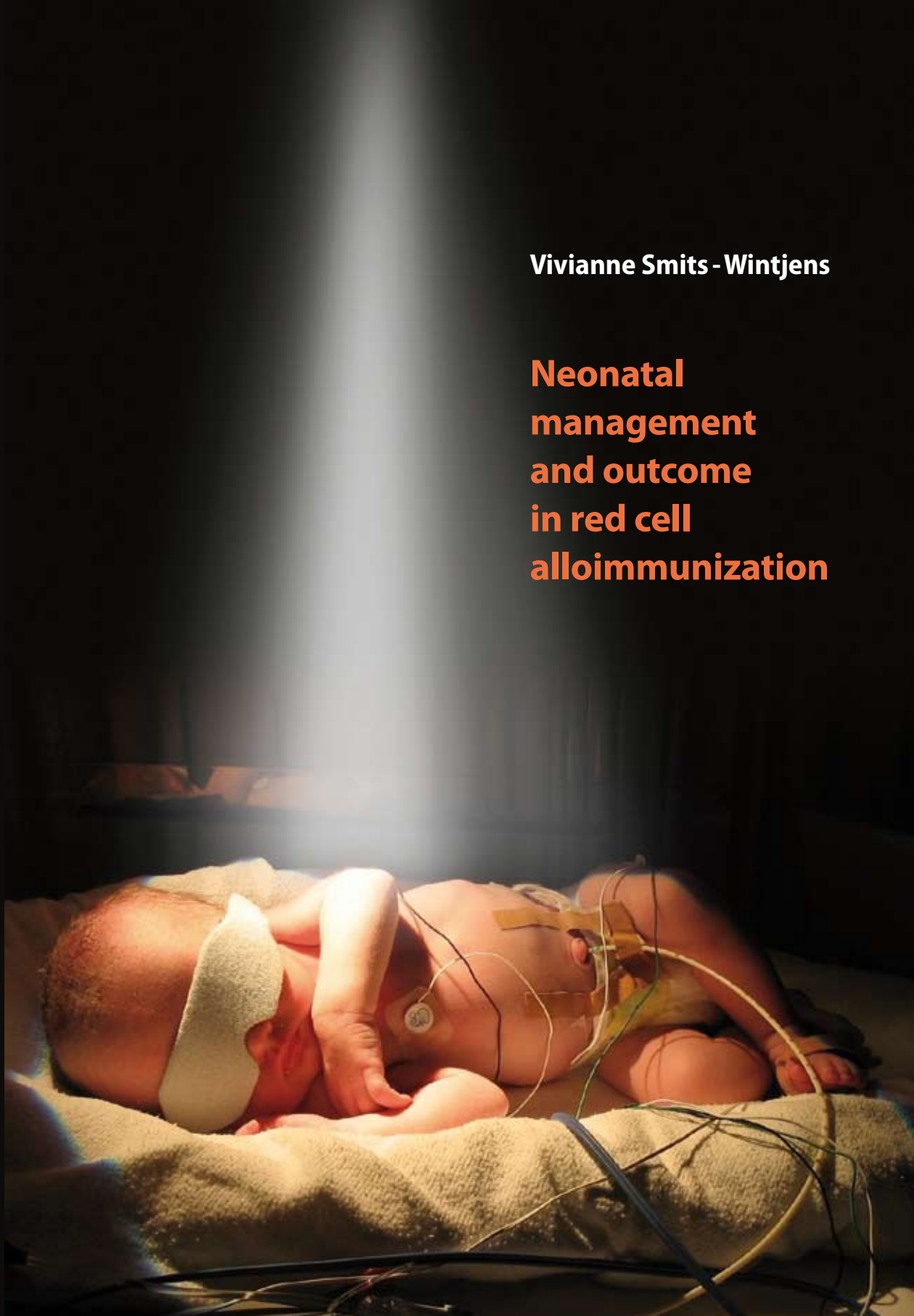

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Aan mijn ouders,
Robert, Koen, Tijn en Lieke

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1

General introduction and outline of the thesis

Introduction

Hemolytic disease of the fetus and newborn (HDFN) due to red cell alloimmunization has been a fascinating clinical picture for many centuries. The first report of a condition called hydrops fetalis dates back to 1609,[1,2] when a French midwife described the delivery of twins. The first twin was hydropic and stillborn and the second suffered from jaundice and subsequently died of kernicterus. These two conditions were not linked until 1932, when Diamond et al. described that hydrops and kernicterus were manifestations of the same disease, which they called erythroblastosis fetalis.[3] However, the exact cause was still unknown. Since 1940, the analyses of Landsteiner and Wiener contributed largely to a better understanding of the pathogenesis of HDFN.[4-7] In their studies with rhesus monkeys Landsteiner and Wiener observed that agglutination of human red blood cells occurred in the presence of rhesus monkey red cell antiserum, whereas subjects who lacked the antigen on their red cells did not show agglutination.[4,7] The authors called this antigen 'Rhesus' (Rh) and consequently the Rh-blood group system was born. Since then it has become clear that the most common cause of severe HDFN is 'Rhesus disease', resulting from maternal immunization to the Rhesus D (Rh D) antigen.[8] However, more than 50 other red cell antigens associated with hemolytic disease have been described. Not only anti-Rh D, but also anti-Rh c and anti-Kell antibodies are associated with severe fetal and neonatal disease.[9]

Untreated HDFN is a major cause of perinatal mortality. In HDFN due to red cell alloimmunization, maternal immunoglobulin (IgG) antibodies directed against fetal red blood cells, pass the placenta into the fetal circulation and cause destruction of fetal red cells. The resulting progressive fetal anemia will then lead to fetal hydrops and perinatal death.[10]

During the last few decades prenatal care for patients with red cell alloimmunization has improved significantly. The introduction of Rh D prophylaxis in the late 1960s,[11,12] the use of Doppler ultrasound to detect fetal anemia since the early 1970s[13] and treatment with intrauterine intravascular red cell transfusions (IUT) since the 1980s[14-16] have led to a remarkable reduction in perinatal mortality. Before the introduction of Rh D prophylaxis and IUTs, perinatal mortality was approximately 50% and the rate of perinatal morbidity was extremely high.[1,17] The current most successful treatment, the use of IUTs, contributed largely to perinatal survival rates exceeding 95% in experienced centers.[10,18] As a result of improving prenatal care strategies and consequently increased perinatal survival, attention is now shifting towards postnatal short-term and long-term management and morbidity.

Neonatal red cell alloimmunization may lead to excessive hyperbilirubinemia and permanent brain damage due to kernicterus. Postnatal management is based mainly on the treatment of hyperbilirubinemia and consists of intensive phototherapy and exchange

transfusion (ET).[18] Despite improvement in neonatal intensive care, ET remains a high risk invasive procedure requiring the use of central lines and is associated with a significant rate of adverse reactions.[19-26] Neonatal treatment with intravenous immunoglobulins (IVIg) has been suggested and used as an alternative therapy for ET.[27] In a few small randomized controlled trials IVIg reduced the need for ET and duration of phototherapy. However, these studies were restricted by several important methodological limitations.[28-31] In 2002 a Cochrane review suggested that more well-designed trials were needed before routine use of IVIg could be recommended for treatment of HDN due to red cell alloimmunization.[32]

Postnatal management also consists of treatment of early and late anemia using top-up red blood cell transfusions.[33] Several risk factors for neonatal anemia secondary to red cell alloimmunization have been reported, including IUT[34], severity of HDN[35,36], type of alloimmunization (including Kell versus Rh D)[37] and the use of ET.[18,38] However, only a limited number of studies (mostly case reports) have been published on differences in type of alloimmunization related to severity of neonatal anemia.[37,39-41] In addition, the protective role of ET for neonatal anemia has only been demonstrated in one small study.[38] Therefore, further research on anemia secondary to red cell alloimmunization is needed.

In the past, various other postnatal complications in neonatal red cell alloimmunization have been reported, including cholestatic liver disease and thrombocytopenia. The etiology of cholestasis in neonates with HDN due to red cell alloimmunization has been attributed to iron overload due to IUT.[42-45] However, data on incidence, potential risk factors, neonatal management and outcome of cholestasis in red cell alloimmunization is scarce. Simultaneously, only a few small studies have been published on incidence and severity of thrombocytopenia in neonates with red cell alloimmunization.[36,46-48] Therefore, more studies are needed to investigate these and other associated complications of neonatal red cell alloimmunization.

As perinatal survival improves, attention is shifting towards long-term outcome in survivors. One of the concerns of the successful use of IUTs is that a decrease in perinatal mortality may lead to an increase in children with long-term handicaps. Moreover, not much is known about the relation between the severity of fetal anemia and long-term neurodevelopmental outcome.[18] To date, only a few studies with small patient numbers have reported long-term neurodevelopmental outcome after IUT.[49-56] Further research on this topic is needed to determine incidence of and risk factors for adverse neurodevelopmental outcome after IUT.

The aim of this thesis was to investigate various management options and to describe complications and outcome of neonatal red cell alloimmune hemolytic disease.

Outline of the thesis

During this study period several study projects on management, complications and outcome in hemolytic disease of the newborn (HDN) due to red cell alloimmunization were performed, including the **L**eiden's **IVI**g trial in Rhesus disease of the **N**eonate (**LIVIN** study) and the **LO**ng-**T**erm follow-up after intra**U**terine transfusion**S** (**LOTUS**) study. The LIVIN study is a randomized controlled trial (RCT) designed in collaboration with Sanquin Blood Bank (Southwest Region, Amsterdam) to determine whether the prophylactic use of IVIg reduces the need for ET in neonates with Rh D or c hemolytic disease. The LOTUS study is a large national cohort study designed in close collaboration with the Department of Obstetrics and the Department of ImmunoHematology and Blood Transfusion of the Leiden University Medical Center (LUMC). One of the aims of the LOTUS study was to evaluate the long-term neurodevelopmental outcome of children treated with IUT. The aims and outcomes of these and other studies are described in the following chapters:

Chapter 2
Review of the literature on HDN due to red cell alloimmunization. This review focuses on postnatal management, associated morbidity and long-term outcome.

Chapter 3
Randomized controlled trial on the use of IVIg in neonates with Rhesus HDN (LIVIN study), investigating the effect of IVIg on number of ETs.

Chapter 4
Case report describing a term neonate with Rhesus hemolytic disease treated with ET and developing a rare but severe cerebral infection after umbilical cord catheterization.

Chapter 5
Study on morbidity associated with ETs in neonatal red cell alloimmune hemolytic disease.

Chapter 6
Study on cholestasis in neonates with red cell alloimmune hemolytic disease, describing incidence, risk factors and outcome.

Chapter 7
Study on thrombocytopenia at birth in neonates with red cell alloimmune hemolytic disease, focusing on incidence and severity of and risk factors for thrombocytopenia at birth.

Chapter 8
Study on top-up red blood cell transfusions in neonates with Rhesus hemolytic disease in relation to ETs.

Chapter 9
Study on neonates with Kell hemolytic disease, focusing on ETs and top-up red blood cell transfusions.

Chapter 10
Study on long-term neurodevelopmental outcome after IUT for fetal alloimmune anemia (LOTUS study), to determine the incidence of and risk factors for neurodevelopmental impairment (NDI).

Chapter 11
General discussion and future perspectives

Chapter 12
Summary

Chapter 13
Samenvatting

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2

Rhesus hemolytic disease of the newborn: postnatal management, associated morbidity and long-term outcome

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Abstract

Rhesus hemolytic disease of the newborn can lead to complications such as hyperbilirubinemia, kernicterus and anemia. Postnatal management consists mainly of intensive phototherapy, exchange transfusion and blood transfusion. During the last decades, significant progress in prenatal care strategies for patients with Rhesus hemolytic disease has occurred. New prenatal management options have led to a remarkable reduction in perinatal mortality. As a result of the increase in perinatal survival, attention is now shifting towards short-term and long-term morbidity. This review focuses on the management of neonatal and pediatric complications associated with Rhesus hemolytic disease, discusses postnatal treatment options and summarizes the results of studies on short-term and long-term outcome.

Introduction

Rhesus hemolytic disease of the newborn (RHDN) results from maternal red-cell alloimmunization. Production of maternal antibodies directed against the fetal red blood cells occurs when fetal red blood cells positive for a certain antigen, usually Rhesus D (Rh D), pass into the blood circulation of a mother negative for that particular antigen. Maternal immunoglobulin (IgG) antibodies might then cross the placenta into the fetal circulation and cause a wide variety of symptoms in the fetus, ranging from mild to severe hemolytic anemia and fetal hydrops. During the last few decades, a significant evolution in prenatal care strategies for patients with RHDN has occurred, including the introduction of Rh D prophylaxis, use of Doppler ultrasound to detect fetal anemia and in particular treatment with intrauterine blood transfusions (IUTs). These new management options have led to a dramatic decrease in perinatal mortality. Before the introduction of Rh D prophylaxis and IUTs, perinatal mortality was approximately 50% and the rate of perinatal morbidity was extremely high.[1,2] With the use of IUTs, overall perinatal mortality in severe RHDN has decreased to less than 10%.[3] As a result of the increase in perinatal survival, attention is now shifting towards short-term and long-term morbidity. Postnatal management of RHDN is based mainly on the treatment of hyperbilirubinemia and consists of intensive phototherapy (PT) and exchange transfusions (ETs) to prevent kernicterus. Various other postnatal complications in RHDN have been reported, including early and late anemia, thrombocytopenia, cholestasis and adverse long-term neurodevelopmental outcome. However, few studies have focused on postnatal management and outcome of RHDN.[2,4-7]

This chapter focuses on the management of neonatal and pediatric complications associated with RHDN, discusses the efficacy of various postnatal treatment options and summarizes the results of studies on short-term and long-term outcome in RHDN.

Management of hyperbilirubinemia and the prevention of kernicterus

RHDN can lead to severe hyperbilirubinemia, acute bilirubin encephalopathy and eventually chronic bilirubin encephalopathy, also known as kernicterus. Prevention of kernicterus is considered to be the primary goal of postnatal treatment of RHDN.[8]

The acute stage of bilirubin encephalopathy is divided into three phases. In the early phase, affected infants become lethargic, hypotonic and suck poorly; the intermediate phase is characterized by stupor, irritability, fever, high-pitched cry and alternating hypertonia

and hypotonia; the advanced phase is characterized by irreversible damage to the central nervous system (CNS) resulting in pronounced retrocollis and opisthotonus, shrill cry, no feeding, apnea, fever, deep stupor to coma, sometimes seizures and death.
The chronic stage of bilirubin encephalopathy, i.e. kernicterus, refers to the clinical CNS findings caused by bilirubin toxicity to the basal ganglia and various brain stem nuclei. In kernicterus, infants develop a severe form of athetoid cerebral palsy, hearing problems and psychomotor handicaps.[8-10]

Phototherapy

Treatment of neonatal hyperbilirubinemia with phototherapy (PT) was introduced in the early 1970s.[8,11] PT lowers serum bilirubin levels through photo-oxidation and converts bilirubin to a water-soluble substance. To deliver conventional PT, the infant is nursed under halogen or fluorescent lamps and the eyes are covered with a mask to prevent retinal damage. However, in the last 10 years new devices for delivering PT have been developed, these are the so-called fibreoptic PT devices. The infant is nursed on a blanket containing optical fibres that deliver light to the back. A recent Cochrane review suggests that even though fibreoptic PT has a place in the management of physiological neonatal hyperbilirubinemia, the use of fibreoptic PT in the treatment of pathological neonatal hyperbilirubinemia due to hemolysis should be investigated in a randomized controlled clinical trial.[11]
The efficacy of PT is dependent on a number of factors, including spectral qualities of the delivered light (optimal wavelength range 400-520 nm), irradiance (intensity of light), surface area receiving PT, distance from the light to the skin (the optimal distance is approximately 30 cm), skin pigmentation, total serum bilirubin concentration at the start of PT and duration of exposure.[4,12,13] PT is required when total serum bilirubin levels exceed the predefined bilirubin thresholds. Recently, new PT guidelines for bilirubin thresholds for children with RHDN have been published by the American Academy of Pediatrics (AAP).[8]

In RHDN, intensive PT should be started immediately after birth, as bilirubin levels may rise sharply after birth. Prompt and intensive PT treatment might prevent the need for ETs. Intensive PT implies the use of high levels of irradiance (430-490 nm, i.e. usually 30 μW/cm^2 per nm or higher) distributed to as much of the infant's surface as possible. In intensive PT the nappy (diaper) should be removed to achieve optimal surface area exposure. Additional surface area exposure can be achieved by lining the sides of the bassinet with aluminium foil or a white cloth. Intensive PT consists of (at least) two PT lamps above and a fibreoptic pad under the infant.[8]

Antenatal treatment with IUTs can reduce the need for PT. In a recent study performed at our center, we compared postnatal outcomes in 89 term and near-term infants with RHDN

treated with and without IUTs.[7] We found a significant reduction in duration of PT in the IUT group: the duration of PT in the IUT and no-IUT groups was 3.8 and 5.1 days, respectively ($p = 0.01$). Replacement of fetal red blood cells by donor adult red blood cells via IUT reduces hemolysis and hence also the need for PT. We found no correlation between the number of IUTs and the duration of PT.

In some forms of RHDN, in particular Kell alloimmunization, anemia is more prominent than hyperbilirubinemia. Kell alloimmunization is the second most common alloimmunization after anti-D and accounts for 10% of the cases of severe hemolytic disease of the neonate. In Kell alloimmunization, a trilineage pancytopenia due to suppressed hematopoiesis is seen. Therefore, antibodies against antigens of the Kell blood group system should be considered as a potential cause of unexplained inhibition of myelopoiesis[14]. Kell alloimmunization might therefore lead to severe fetal and neonatal anemia secondary to bone marrow depression rather than hemolysis. Consequently, only minimal PT might be necessary, despite severe anemia.[4,15,16]

Exchange transfusion

Treatment of neonatal hyperbilirubinemia with exchange transfusion (ET) was introduced in the early 1950s.[17] RHDN is one of the most common indications for ET. ET prevents kernicterus by removing bilirubin from the circulation. In infants with RHDN, ET has the additional benefits of removing maternal antibodies (thus limiting further hemolysis) and correcting the associated anemia. ETs are performed with double volume transfusion (160 mL/kg) using irradiated, leukocyte-depleted erythrocytes (cross-matched against the mother and compatible with the infant) via an intravenous catheter, usually an umbilical vein.

ET is required in RHDN when intensive PT management fails and serum bilirubin levels approach the threshold for ET. The percentage of children with RHDN requiring treatment with ET ranges from 20% to more than 70%.[7] This wide range is partly due to the use of different serum bilirubin thresholds for ET. Comparison of the incidence of ET in children with RHDN between the various studies is therefore difficult to accomplish. Several studies mandated the use of early ET, resulting in a higher rate of ET. Until 2005, management guidelines for RHDN at our center also mandated the use of early ET. The incidence of ET in term infants with RHDN delivered at our center before 2005 was 69%.[7] Most ETs (88%) were performed within the first 12 h of birth. In 2004, the AAP published new ET guidelines and serum bilirubin thresholds for children with RHDN.[8] These new guidelines do not recommend the use of early ET. Our center adopted these new guidelines in 2005 and the incidence of infants with RHDN requiring treatment with ET dropped significantly from 69% to less than 20% thereafter.

Whether treatment with IUT also reduces the need for ET in infants with RHDN is not clear. Neonates treated with IUT have a high percentage of adult (donor) red blood cells and a lower percentage of fetal red blood cells,[18] which should result in reduced hemolysis and less need for ET. In a recent retrospective study performed at our center, we compared the need for ET in infants with RHD treated with or without IUT.[7] We found that the median number of ETs was similar in the IUT and no-IUT groups. However, this paradoxical result might be related to the specific early ET criteria used in this study.[7,19,20]

Despite improvement in neonatal intensive care, ET still remains a high-risk invasive procedure associated with a significant rate of adverse reactions. Although the mortality rate associated with ET is nowadays less then 0.3% in term infants, morbidity rates may reach 24%.[21-25] Morbidity associated with ET includes, in particular, catheter-related complications. Umbilical venous catheterization is often required to perform an ET. Although umbilical venous catheterization is a common procedure in the management of sick neonates, it can lead to serious complications, including dislodgement, thrombosis, hemorrhage, arrhythmias, (pericardial) effusions, portal hypertension and infection.[25-30] The incidence of sepsis related to umbilical venous catheterization is reported to range between 6% and 24%.[31]

Intravenous Immunoglobulin

The new AAP guidelines recommend the use of 0.5–1.0 g/kg intravenous immunoglobulin (IVIg) in RHDN in case of failure of PT to reduce the need for ET.[8] The exact mechanism for IVIg in RHDN is yet incompletely unraveled and is the subject of ongoing research. IVIg might increase IgG catabolism, resulting in a shorter half-life of antibodies (including anti-Rh antibodies); IVIg might also block the IgG-receptor on macrophages, resulting in a decreased removal of anti-Rh-coated erythrocytes from the circulation. A third hypothesis is the presence of anti-idiotypic antibodies as a result of IVIg treatment neutralising anti-Rh antibodies.[32]

A few small, randomized controlled trials have suggested that IVIg combined with PT reduces serum bilirubin levels and the need for ET in neonates with RHDN compared with PT alone. In these studies, treatment with IVIg also reduced the duration of PT and length of hospitalization, but increased the need for late red blood cell transfusions. However, the number of patients included in these randomized controlled trials was small and the study-design and inclusion criteria varied considerably. One study included infants with ABO incompatibility;[33] an unexpected and large number of these children with ABO incompatibility and hemolytic disease required an ET. Finally, the criteria for ET were discordant between the various studies.[33-36] A recent Cochrane review suggested that the results of further trials of higher quality should be awaited and stated that: "further well designed studies are needed before

routine use of IVIg can be recommended for the treatment of isoimmune hemolytic jaundice".[37] In view of this dilemma, we recently started a prospective, randomized double-blind, placebo-controlled trial to assess the short- and long-term effects of the prophylactic use of IVIg in neonates with RHDN who were delivered at our center (the LIVIN study: http://www.controlledtrials.com/ISRCTN14013064/). A total of 40 patients is required in both arms of the study to detect a reduction in ETs from 30% in the placebo group to 6% in the IVIg group. To date, 38 patients have been enrolled, and final results will be awaited by the end of 2009.

Albumin

Bilirubin is transported in the plasma bound to albumin. The fraction of bilirubin that is not bound to albumin can more readily cross the blood-brain barrier and may cause bilirubin encephalopathy. The recent AAP guidelines suggest that serum albumin levels should be measured routinely, as an albumin level of less than 3 g/dL can be considered as a risk factor for lowering the threshold for PT.[8] The AAP also recommends that if an ET is being considered, the bilirubin/albumin ratio should be used to determine the need for an ET. Administration of albumin before ET may increase the efficacy of the ET, because more bilirubin will be mobilised from the tissues into the blood and excreted. However, evidence that albumin infusion increases the long-term outcome in infants with severe hyperbilirubinemia is not available and thus routine use of albumin is not recommended.[8]

Phenobarbital

Phenobarbital increases bilirubin uptake, conjugation and excretion. A few studies in the 1970s and 1980s suggested that administration of phenobarbital at birth to a child with hyperbilirubinemia might decrease the need for ET.[38-40] However, recent studies suggest that postnatal administration of phenobarbital does not offer additional advantage over routine use of intensive PT.[5,41]

A recent retrospective study by Trevett et al. showed that antenatal maternal administration of phenobarbital significantly reduces the need for ET in neonates affected with RHDN.[42] The incidence of ET in neonates with and without antenatal phenobarbital administration was 9% versus 52%, respectively ($p < 0.01$). As suggested by the authors, further study in a randomized controlled trial is necessary to confirm these results.[42]

Metalloporphyrins

In recent years, various metalloporphyrins (also known as heme oxygenase inhibitors) have been used to prevent and treat unconjugated hyperbilirubinemia. Metalloporphyrins act by inhibiting the enzyme heme oxygenase, the rate-limiting step in the catabolism of heme to bilirubin. Metalloporphyrins are natural or synthetic heme analogues, which reduce the

production of bilirubin. By preventing the formation of bilirubin, metalloporphyrins have the potential to reduce the level of unconjugated bilirubin and thus decrease the need for PT and hospitalization. However, routine treatment is not recommended at present. A recent Cochrane review suggests that randomized controlled trials are required to compare metalloporphyrin treatment with placebo and to report on important outcomes such as severe hyperbilirubinemia, neonatal kernicterus, ET and long-term neurodevelopmental impairment.[5,43,44]

Hydration

PT increases insensible water loss through the skin and raises the fluid requirements of infants undergoing PT.[45-47] In addition, by-products of PT are eliminated in the urine. If oral hydration is inadequate, intravenous hydration may be necessary.[48] However, there is no unequivocal evidence that increased fluid administration affects serum bilirubin concentration. An exception should be made for infants who are dehydrated, as they might need supplemental fluid intake to correct their dehydration. For breastfed infants with evidence of dehydration, supplementation with a milk-based formula inhibits the enterohepatic circulation of bilirubin and can improve the efficacy of PT.[13]

Management of anemia

Anemia may be present at birth (early anemia) or not until 1 - 3 weeks of age (late anemia). The degree of anemia varies in infants with RHDN. Late (hyporegenerative) anemia presenting 1 week - 3 months after birth is a common problem in neonates with RHDN. Late anemia in RHDN is characterised by a reduction in reticulocyte count and low serum erythropoietin (EPO) levels.[49] Other causal factors include reduction of the half-life of the transfused erythrocytes in infants who received IUTs and red blood cell transfusion or ET postnatally.[7,49-52]

The incidence of late anemia in neonates with RHDN ranges from 71 to 83%.[7,49] Late anemia in RHDN usually resolves by the third month of life.[5,7] In a recent study performed at our center we found that neonates with RHDN treated with IUTs required more top-up red cell transfusions during the first 6 months of life than neonates with RHDN not treated with IUTs (77% and 26.5% respectively; $p < 0.01$). Infants treated with IUTs had a significantly lower median reticulocyte count at birth than infants without IUT (7 ‰ versus 73 ‰, respectively; $p < 0.01$). The association between low reticulocyte count and increased need for top-up transfusions indicates that IUTs could result in suppression of erythropoiesis and in bone marrow hypoactivity.

Infants with RHDN treated with IUT must receive irradiated blood transfusions to prevent

the risk of transfusion associated graft-versus-host disease. Infants must be checked for the rate of hemoglobin fall once or twice a week (depending on the level of the hemoglobin concentration) until 3 months of age. However, international guidelines for red blood cell transfusion in infants during the first months after birth are not available and consensus on appropriate transfusion triggers and the volume of blood to be transfused is not available. Most countries use a transfusion protocol based on clinical condition, mechanical ventilation, gestational age, oxygen use and hematocrit or hemoglobin levels. In our center term neonates with RHDN are treated with transfusions of red blood cells when hemoglobin levels fall below 8.0 g/dL (5.0 mmol/L) or below 9.6 g/dL (6.0 mmol/L) when clinical symptoms of anemia are present (need of extra oxygen, poor feeding, tachycardia and/or tachypnoea).

Erythropoietin

Erythropoietin (EPO) can be used to prevent late anemia and reduce the need for top-up transfusions of red blood cells. Recently, several studies and meta-analyses of EPO administration showed insufficient evidence to comment on the possible advantages of EPO. Routine use of EPO in infants with late anemia due to RHDN is therefore not recommended.[53-56]

Folic acid

The administration of folic acid until 3 months of age might - hypothetically - decrease the need for top-up transfusions of red blood cells by stimulating erythropoiesis. Suggested dosages vary from 25 to 1000 µg daily. However, there are no available data in the literature to support or refute this hypothesis. Current studies do not provide any evidence that administration of folic acid reduces the need for top-up transfusions of red blood cells. The available data are therefore insufficient to directly guide routine clinical practice.[51]

Iron

As discussed above, neonates with RHDN often require IUTs and (multiple) transfusions of red blood cells. The risks and potential consequences of iron overload due to these multiple transfusions are poorly recognized. High levels of cord blood ferritine have been reported in infants with RHDN.[57] As infants with RHDN already have high iron storage, supplementation of iron is not recommended and should not be used.[57]

Management of other associated morbidity

Hydrops fetalis

Severe fetal anemia in RHDN can lead to hydrops fetalis. The immediate postnatal management of a newborn with hydrops fetalis constitutes one of the major challenges in neonatal medicine. Newborns with RHDN and hydrops fetalis have generalized subcutaneous edema and – often – fluid collections in pericardial, pleural or peritoneal spaces. Hydropic infants tolerate labour poorly and are usually depressed at birth. Intubation is often required and can be difficult because of edema. High pressures might be required during mechanical ventilation because of pulmonary edema and pulmonary hypoplasia (secondary to pleural effusions and ascites). Immediate drainage of ascites and pleural effusions in the delivery room might be life-saving procedures. If pulmonary hypertension induces severe hypoxemia, other treatment options should be envisaged, such as mechanical ventilation with high-frequency ventilation, inhaled nitric oxide or extra corporal membrane oxygenation. Circulatory insufficiency is often present and requires adequate inotropic support. As newborns with fetal hydrops frequently show signs of cardiac decompensation, correction of chronic anemia should preferably be performed with a partial ET rather than with a simple transfusion accompanied by administration of diuretics.[58]

Thrombocytopenia

Recent literature suggests an association between low fetal platelet counts and fetal hydrops in severe Rh D alloimmunized pregnancies.[59-61] Fetal thrombocytopenia may have grave consequences, such as intracranial hemorrhage and prolonged, possibly life-threatening bleeding from the puncture site of the IUT. In a recent study performed at our center, fetal platelet counts were measured prior to 914 IUTs in Rh D alloimmunized pregnancies. Severe fetal thrombocytopenia (platelet count < $50x10^9$/L) was found in 3% of all fetal blood samplings and in 23% of severely hydropic fetuses. Perinatal mortality in fetuses with severe thrombocytopenia was 36% (van den Akker, personal communication). The incidence and severity of thrombocytopenia during the neonatal period is not known.

Cholestasis

Several case reports describe elevated levels of conjugated bilirubin in neonates with RHDN.[8,62-65] The incidence and pathogenesis of cholestasis and bronze baby syndrome in newborns with RHDN is not known. Cholestatic liver disease in neonates with RHDN treated with multiple IUTs, red blood cell transfusions and/or ETs can result from hyperferritinemia and liver iron overload.[8,62-65] Accumulation of copper porphyrines in serum and tissues, found in bronze baby syndrome, can be responsible for a lower plasma albumin concentration and less binding of bilirubin, posing a greater risk for kernicterus in patients with bronze baby syndrome.[64]

The current AAP guidelines state that thresholds for the treatment of hyperbilirubinemia should be based on the total serum bilirubin levels, without subtracting the conjugated bilirubin fraction. Nevertheless, when conjugated bilirubin is significantly elevated (i.e. > 50%) there is no bilirubin threshold at which intervention is recommended. Bilirubin encephalopathy is due to unbound, unconjugated bilirubin. Whether neonates with elevated conjugated bilirubin are at increased risk for kernicterus is not known and management remains controversial. Treatment should ideally be based on the measurement of unbound, unconjugated bilirubin, but these measurements have not yet been adapted for clinical use.[8,62-65]

Long-term neurodevelopmental outcome and morbidity

Most study groups, including ours, nowadays report perinatal survival rates in red blood cell alloimmunization treated with IUTs above 90%.[3] As perinatal survival improves, attention is shifting towards long-term outcome in survivors. To date, only a few small studies have reported on the long-term neurodevelopmental outcome in RHDN. The main limitation of these studies is the small number of patients included (range 16-69). Moreover, although hydrops fetalis is associated with increased mortality, not much is known about the association between the severity of fetal anemia and long-term neurodevelopmental outcome.[66-70] Doyle et al. reported on the sensorineural outcome at 2 years of age in 38 survivors of fetal IUTs. The majority of these infants (92%) showed no sensorineural disability at 2 years of age.[66] In a follow-up study performed at our center, Janssens et al. found that the neurodevelopmental outcome for children with RHDN treated with IUTs compared favorably with a group of high-risk, very-low-birth- weight infants (10% versus 18%, respectively) and less favorably with a healthy control group (10% versus 6%, respectively).[70] Hudon et al. studied the neurodevelopmental outcome in 40 infants with RHDN treated with IUT. All infants showed normal developmental outcome at the age of 62 months.[69] Grab et al. described 35 infants treated with IUTs for severe erythroblastosis. At 6 years of age no moderate or severe neurologic impairment was observed.[68] Harper et al. evaluated long-term outcome in 18 hydropic fetuses treated with IUT. Death or major neurological morbidity occurred in 22% of the fetuses and 12% of the survivors had major neurologic sequele.[67]

In 2008, we will perform a follow-up study of all the children (n=350) with RHDN treated with IUTs at our center between 1992 and 2007. Our primary objective is to assess long-term neuromotor development, cognitive development and psychosocial well-being in the largest cohort reported so far. Our secondary objective is to investigate the association between adverse long-term outcome with risk factors, including gestational age at birth, cause and severity of fetal anemia, presence and severity of hydrops fetalis, and number of IUT procedures. Results of this study will be available by 2010.

Practice points

- New guidelines for PT and ET thresholds have been published recently by the AAP.
- The new AAP guidelines do not recommend the use of early ET.
- Supplementation of iron in infants with RHDN is not recommended in consideration of the high iron storage.

Research agenda

- Well-designed randomized controlled trials are necessary to address the efficacy and safety of IVIg use in newborns with RHDN.
- Randomized controlled studies in RHDN are required to evaluate the efficacy of various interventions, such as EPO and folic acid administration, to reduce the need of top-up red blood cell transfusions.
- Short-term outcome studies in infants with RHDN treated with IUTs are needed to investigate the incidence of associated morbidities, including cholestasis.
- Long-term follow-up studies in large cohorts of infants with RHDN treated with IUTs are required to determine their neurodevelopmental outcome and determine potential risk factors.

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

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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 randomized for IVIg (0.75 g/kg) or placebo (glucose 5%). Primary outcome was the rate of exchange transfusions. Secondary outcomes were duration of phototherapy, maximum bilirubin levels and the need of 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% (7/41) versus 15% (6/39), p=1.00) and in number of exchange transfusions per patient (median (range) 0 (0-2) versus 0 (0-2), p=0.90), nor 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% (34/41) versus 87% (34/39), p=0.76).

Conclusion: Prophylactic IVIg does not reduce the need for exchange transfusion nor 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 a RCT to address this question.

Materials and Methods

We performed a prospective 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 (Rh D) or c antibodies in the fetus/neonate of a Rh D 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 Rh D 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 of 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 of 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).

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.

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

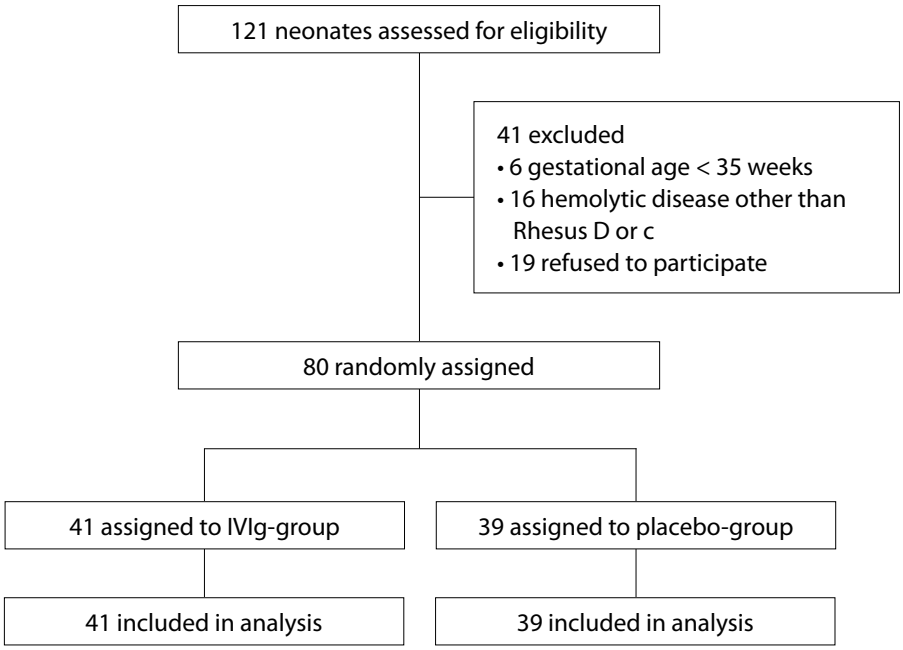

Figure 1 Flow diagram of study participants

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)

Table 2 Neonatal outcome 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 ET-n (%)	7 (17)	6 (15)	0.99	7 (26)	4 (15)	0.50	0 (0)	2 (15)	0.22
Number of ETs per neonate [b]	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 [a]	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 [a]	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 [a]	7 ± 4	7 ± 3	0.37	6 ± 4	7 ± 3	0.58	7 ± 4	8 ± 3	0.45

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

Table 3 Top-up transfusions in neonates with Rh 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 [b]	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 (26)	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 [a]	12 ± 12	16 ± 15	0.24	12 ± 12	16 ± 16	0.33	13 ± 11	17 ± 15	0.50
Hb level at first top-up transfusion–g/dL [a]	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 mean ± SD
[a] Value given as median (range)

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 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.

Comment

In this RCT we have shown that prophylactic treatment with IVIg in neonates with Rhesus hemolytic disease did not reduce the need for ET nor 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 a 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 nor 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 subgroup 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 inter-

preted 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]

Conclusion

Prophylactic treatment with IVIg (in a dosage of 0.75 g/kg) did not reduce the need for ET nor 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.

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4

Bacillus cereus cerebral abscesses in a term neonate with Rhesus hemolytic disease treated with exchange transfusion

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Abstract

Exchange transfusion (ET) is the most effective method for treatment of severe hyperbilirubinemia and is often required in Rhesus hemolytic disease of the newborn (RHDN). The use of ET is also associated with adverse reactions, including severe catheter-related infectious complications. We report a term neonate with RHDN treated with an ET through an umbilical venous catheter who developed brain abscesses due to a Bacillus cereus sepsis. This severe complication has not previously been reported. We discuss possible causes for this severe infection and provide suggestions on prevention.

Introduction

Exchange transfusion (ET) plays an important role in the treatment of hyperbilirubinemia of the newborn. Rhesus associated hemolytic disease of the newborn (RHDN) is one of the most common indications for ET. The main therapeutic effect of ET is removal of excess bilirubin and maternal red blood cell antibodies in order to prevent kernicterus and to reduce hemolysis. Despite improvement in neonatal intensive care, ET still remains a high-risk invasive procedure associated with a significant rate of adverse reactions. Although the mortality rate associated with ET is nowadays less then 0.3% in term infants, morbidity rates may reach 5%.[1] Morbidity includes in particular catheter-related complications such as thrombosis, sepsis and necrotizing enterocolitis.[1]

We report a term neonate with RHDN treated with an ET who developed a rare but severe cerebral infection after umbilical cord catheterization.

Case report

A 36-year-old gravida 2 para 1 with severe Rh D alloimmunization required 3 intrauterine transfusions with filtered red blood cell concentrate for fetal anemia. These procedures were uncomplicated. The last transfusion before birth was done at 34 weeks' gestation, with a post-transfusion hemoglobin level of 15.1 g/dl. The first labour had been protracted and was complicated by a complete perineal laceration after vacuum assisted delivery. For this reason the parents required a caesarean delivery, which was planned 3 weeks after the final intrauterine transfusion at a gestation of 37 weeks. A male infant was born with a birth weight of 2400 grams (25th percentile for gestational age) and Apgar scores of 10, 10 and 10 at 1, 5 and 10 min, respectively. No abnormalities were detected on physical examination, except a pale skin color. Laboratory investigations at birth showed a hemoglobin value of 9.6 g/dL, a total bilirubin concentration of 11.8 mg/dL and a conjugated bilirubin concentration of 11.3 mg/dL. Treatment with intensive phototherapy was started immediately after birth. After obtaining parental informed consent, the infant was included in a randomized placebo controlled trial to assess the short and long term effects of prophylactic use of intravenous immunoglobulin (IVIg) in neonates with RHDN (LIVIN trial: http://www.controlled-trials.com/ISRCTN14013064/). For this study a ready for use 5% IVIg solution (Nanogam® Sanquin, The Netherlands) or glucose 5% (placebo) was used. Our patient was randomized for IVIg which he received within four hours after birth. Due to persisting elevated bilirubin concentrations and possible need for an ET an umbilical venous catheterization was performed. Despite intensive phototherapy, the total serum bilirubin

level increased gradually to a maximum level of 21.2 mg/dL, exceeding the threshold for ET, which was performed on day 3 through the umbilical vein catheter.

On day 6, the infant became acutely ill, his body temperature rose to 39.0°C and he became irritable. The white blood cell count was 8,100/μL (with 10% band forms) and the C-reactive protein concentration was 59 mg/L. Cerebrospinal fluid (CSF) analysis showed pleocytosis (10,513 cells/μL) and an elevated protein content (137 mg/dL), but the volume of CSF was not sufficient for culture. A peripheral blood culture was obtained and processed according to standard protocol, using culture vials for pediatric blood specimens (BACTEC culture vials type PED PLUS™ /F, enriched soybean-casein digest broth with resins). Meningitis therapy was initiated with vancomycin and ceftazidim intravenously. Subsequently, a lumbar puncture was repeated and CSF was collected for culture. Urinalysis was normal. On day 7, the blood culture came back positive (after 24 h incubation) for a Gram-positive, rod-shaped, beta-hemolyticum bacterium, identified as Bacillus cereus. It was found susceptible to vancomycin, meropenem and clindamycin, but resistant to penicillin. Bacterial and viral CSF cultures remained negative. Cultures of the packed cells used for the intrauterine blood transfusions and for the ET were sterile.

Cranial ultrasound on day 7 revealed a lesion in the right frontal region with signs of central necrosis and a rim of hyperechogenicity around it, suspect for a cerebral abscess, whereas a cranial ultrasound performed on day 1 had shown no abnormalities.
Monotherapy with meropenem was started because of a better passage through the blood-brain barrier. Magnetic resonance imaging (MRI) on day 11 showed multiple hemorrhagic cerebral abscesses in the right frontal, temporal and occipital lobe (Figure 1) and a few small abscesses in the cerebellum.

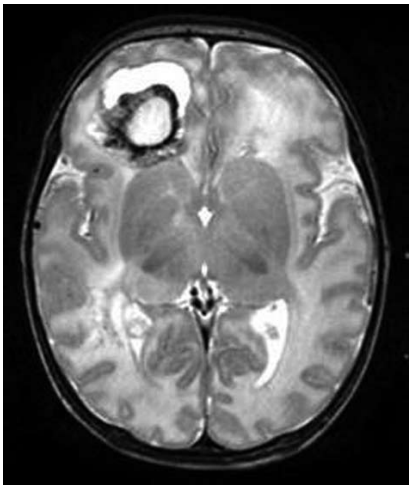

Figure 1 T2-weighted MRI showing a large hemorrhagic abscess in the right frontal lobe

On day 21 the child again became irritable and developed a seizure (stretching of both arms and legs). Because of the short duration of the seizure (less than 1 min) and a normal electroencephalogram (EEG), anticonvulsant medication was not required. On day 29 the infant had a recurrent generalized convulsion and anticonvulsant treatment (phenobarbital) was initiated. Again, the EEG showed no epileptic activity. A MRI performed a few days after the seizures showed no changes in the cerebral lesions.

After 6 weeks of intravenous meropenem administration, antibiotic treatment was discontinued. The infant was discharged home on day 61 with anticonvulsant therapy. At 2 years of age, the infant had no neurologic sequelae on physical examination. Mental and psychomotor development indexes, assessed at 2 years of age with the Bayley Scales of Infant Development, were within normal ranges.

Discussion

ET has been used in neonatal intensive care units for more than 50 years and is still the most effective method for treatment of severe hyperbilirubinemia. However, ET is also associated with severe adverse reactions, including catheter-related infectious complications.[2] We report a term infant with cerebral abscesses due to a Bacillus cereus sepsis, a severe complication which has not previously been reported.

Bacillus cereus is a probably ubiquitous soil bacterium and an opportunistic pathogen that is a common cause of food poisoning. In (premature) neonates, toxins produced by Bacillus cereus can cause necrosis of infected tissue. Bacillus cereus infections in neonates include meningoencephalitis and brain abscesses, characterized by liquefactive necrosis. A few sporadic cases have been reported in the literature and usually involved premature infants, suggesting an association with decreased immune response. Most cases of neonatal meningoencephalitis and cerebral abscesses with Bacillus cereus described in the literature resulted in neonatal death.[3-7]

Several potential risk factors may have led to the Bacillus cereus infection in this infant. Because of severe RHDN, three intrauterine blood transfusions and one ET were required. Multiple blood transfusions are associated with an increased risk of infection. However, cultures of all donor blood used for the transfusions were sterile. Intrauterine transfusions have also been associated with several procedure-related complications, including, although rarely, intrauterine infections. In the largest published series on complications of intrauterine transfusions, only two infections with E. Coli, both resulting in fetal distress within 48 h

after the procedure, were found in 740 procedures.[8] In the current case, no signs of infection were present prenatally, and there was no premature rupture of membranes prior to the elective caesarean section. It seems therefore highly unlikely that the Bacillus cereus infection had a prenatal origin. Moreover, cranial ultrasound on day 1 showed no abnormalities, excluding a link between the cerebral abscesses and antenatal invasive procedures.

Although very unlikely, another potential cause for infection in this case could be the IVIg solution. All IVIg preparations are routinely tested for sterility. The production of IVIg is an aseptical process. Up till now more than 80 batches resulting in approximately 100.000 vials of Nanogam® have been produced. Sterility tests on all these batches were negative.

Lastly, the most probable cause for infection in this case was the umbilical venous catheterization. Although umbilical venous catheterization is a common procedure in the management of sick neonates, it can lead to serious complications. Sequelae include thrombosis, embolization, hemorrhage, arrhythmias, (pericardial) effusions, portal hypertension and infection.[9] In the literature the incidence of sepsis related to umbilical venous catheterization is reported between 6% and 24%.[2]

Umbilical venous catheterization was required in this case because an ET was deemed necessary to reduce the elevated bilirubin levels and the risk of bilirubin encephalopathy. However, whether catheterization and ET was strictly unavoidable is questionable.

First, hyperbilirubinemia was mainly due to elevated levels of the conjugated bilirubin fraction. Elevated levels of conjugated bilirubin are not a rare phenomenon in neonates with RHDN, although the etiology is not clear. The current AAP guidelines state that thresholds for treatment in hyperbilirubinemia should be based on the total bilirubin levels, without subtracting the conjugated bilirubin fraction.[1] Nevertheless, when conjugated bilirubin is significantly elevated (i.e. > 50%) there is no bilirubin threshold at which intervention is recommended. Bilirubin encephalopathy is due to unbound, unconjugated bilirubin. Whether neonates with increased conjugated bilirubin are at increased risk for kernicterus is not known and management remains controversial. Management should ideally be based on the measurement of unbound, unconjugated bilirubin, but these measurements have not yet been adapted to clinical use.

Second, the AAP guidelines recommend the use of IVIg in case of failure of intensive phototherapy in order to reduce the need for ET.[1] The evidence to recommend routine prophylactic treatment with IVIg is considered insufficient.[10,11] A recent Cochrane review suggests that the results of further trials of higher quality should be awaited.[10] Although the risk for

adverse effects of umbilical venous catheterization and ET may decrease in the future, it will never completely disappear. The best way to prevent procedure-related complications in RHDN is to reduce the need for ET.

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5

Neonatal morbidity after exchange transfusion for red cell alloimmune hemolytic disease

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Abstract

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

Patients and Methods: All neonates with HDN 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 during admission (ET-group) and 61% (213/347) did not require ET (no-ET-group). Comparison between the 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 group treated with ET.

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

Introduction

Hemolytic disease of the newborn (HDN) 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[1-3] neonatal treatment with ET became one of the most commonly performed neonatal 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. Several studies have reported on mortality and morbidity rates associated with ET. Although the contemporary mortality rate is reported to be less than 2%, rates of morbidity and ET-related adverse events can reach 74%.[4-11] Reported adverse events include mainly catheter-related complications (malposition, sepsis), complications related to the use of blood products (thromboembolization, graft versus host reactions, infection), metabolic derangements (acidosis, disturbance of serum levels of sodium, calcium, potassium and glucose) and cardio-respiratory reactions (including cardiac arrhythmias, cardiac arrest and apnea).[4-11]

In nearly all previous studies a heterogeneous group of infants with HDN treated with ET was included, varying from red cell alloimmunization to ABO-incompatibility.[4-6,8,10,11] 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, including anemia, idiopatic hyperbilirubinemia and metabolic/intrinsic erythrocyte disorders (pyruvate kinase deficiency, glucose-6-phosphate-dehydrogenase deficiency and Gilbert's disease).[4,10,11] In addition, most studies were limited by a relative small number of included patients.[4-11]

The aim of this study was to evaluate the type and rate of complications associated with ET in a large series of neonates with HDN due to red cell alloimmunization exclusively.

Patients and methods

All term and preterm neonates with HDN 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 red cell alloimmunization in the Netherlands. Neonatal outcome in part of this group was reported in previous studies.[12-16]

Guidelines for the management of neonates with HDN admitted to our nursery (including intensive phototherapy and exchange transfusion) have previously been described.[12,13] The guidelines for ET used in our neonatal center were revised 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/h 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.[14] In December 2005 a new guideline of the American Academy of Pediatrics (AAP) with higher bilirubin thresholds for phototherapy and ET was implemented in our nursery.[17] The criteria for ET after December 2005 were: (1) total serum bilirubin above (higher) ET thresholds[17] 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.

ET was performed with double-volume transfusion (160 mL/kg). In our center ET was performed with heparinized blood until December 2000. As of January 2001 heparinized blood was replaced by citrated plasma from a non-transfused male donor, lacking irregular erythrocyte antibodies, which was added to irradiated, leukocyte-depleted ($< 1\times10^6$) and thrombocyte-reduced red cell concentrate, compatible with maternal antibodies. Citrated plasma contains nearly no free calcium and physiologic levels of potassium and glucose, with an increased concentration of sodium (168 mEq/L). Because of the well-known differences in complications between heparinized and citrated blood,[18,19] we started including patients from the moment citrated plasma was used (January 2001).

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).

An adverse event was defined as any complication that occurred during admission. The following events were recorded (definitions are described between brackets): hypocalcemia (total serum calcium < 8 mg/dL), hypoglycemia (serum glucose < 46.8 mg/dL), hyperkalemia (serum potassium > 6.5 mEq/L), hypokalemia (serum potassium < 3.0 mEq/L), hypernatremia (serum sodium > 150 mEq/L), hyponatremia (serum sodium < 130 mEq/L), metabolic acidosis (requiring treatment with bicarbonate), respiratory failure (requiring respiratory support with continuous positive airway pressure (CPAP) and/or mechanical ventilation), apnea (cessation of respiration for > 20 seconds), pulmonary hemorrhage, cardiac arrest (sudden cessation of heartbeat and cardiac function treated with cardiac resuscitation with either epinephrine and/or chest compressions), hypertension (high blood pressure requiring treatment with antihypertensive medication), hypotension (low blood pressure requiring treatment with intravenous fluids or vasopressors), necrotizing enterocolitis

(classified according to Bell's criteria[20]), proven sepsis (clinical and/or biochemical signs of infection with a positive blood culture), suspected sepsis (clinical and/or biochemical signs of infection without a positive blood culture), disseminated intravascular coagulation (DIC) (requiring treatment with fresh frozen plasma), seizures (clinical evidence of seizure-like activity treated with anti-epileptic medication), leukocytopenia (leukocyte count in the first 24 hours after birth < $9x10^9$/L and after 24 hours < 5×10^9/L)[21], thrombocytopenia (platelet count < 150×10^9/L, defined as severe if platelet count < 50×10^9/L and very severe if platelet count < 20×10^9/L), intraventricular hemorrhage (classified according to Volpe[22]) or other cerebral hemorrhage and neonatal death. In our center, platelet transfusions are given when platelet counts fall below the following thresholds: 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, occurrence of the above mentioned complications during admission and time of occurrence in relation to ET, number of ETs and presence of umbilical venous catheter.

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 < .05 was considered to indicate statistical significance. Of all statistically significant complications identified with univariate analysis between the ET-group and no-ET-group a multivariate logistic regression analysis was performed to measure the independent effect of ET(s). The results of the 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 HDN due to red cell alloimmunization were admitted to our neonatal nursery. We excluded one preterm neonate (born at 29 weeks' gestation) because he died immediately after caesarean section due to severe perinatal asphyxia following unsuccessful IUT. A total of 347 patients were included in this study, 134 (39%) in the group with at least one ET during admission (ET-group) and 213 (61%) in the group without ET (no-ET-group). Baseline characteristics of both groups are summarized in

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
Birth weight - kg[a]	2.9 ± .6	2.9 ± .6	0.591
Gestational age at birth - weeks[b]	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[a]	11.6 ± 2.6	12.7 ± 2.9	< 0.001
Reticulocyte count at birth – ‰[b,c]	49 (6.8-83.3)	39 (3-75.5)	0.089
Leukocyte count at birth – 10^9/L[a,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[a]	7.1 ± 3.1	4.8 ± 2.3	< 0.001
Umbilical venous catheter – n (%)	133 (99)	64 (30)	< 0.001
Days of admission[a]	6.6 ± 3.8	6.2 ± 3.9	0.009

[a] Value given as mean ± SD
[b] Value given as median (IQR)
[c] assessed in 78/134 and 149/213 neonates
[d] assessed in 130/134 and 207/213 neonates

Table 1. The mean (± SD) number of ETs in the ET-group was 1.55 ± 1.01 (median 1, range 1-9). Neonates with Rhesus D type of red cell alloimmunization were more likely to require treatment with ET (91% (122/134) versus 65% (138/213), p < 0.001), whereas treatment with ET was only sporadically required in neonates with Kell alloimmunization (1% (1/134) versus 22% (46/213), p < 0.001).

Complications

Detailed information on complications during admission in the ET-group and the no-ET-group is summarized in Table 2.

Univariate logistic regression analysis

Metabolic derangements/complications

Two metabolic complications had a significantly higher incidence in the ET-group than in the no-ET-group: hypocalcemia (22% versus 1%, OR 29.1, 95% CI 6.8-124.5) and hypernatremia (8% versus 0%, OR not calculated, p < 0.001). Four of 31 (13%) neonates with hypocalce-

mia needed calcium replacement therapy and 3 of 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 between the two groups in respiratory support, apneas, cardiac arrest, and hypotension (Table 2). No cases of cardiac rhythm disorders, pulmonary hemorrhage and hypertension (requiring treatment) occurred.

Infectious complications

Proven sepsis occurred significantly more often in the ET-group than in the no-ET-group (8% versus 1%, OR 6.3, 95% CI 1.7-22.9). In the 14 neonates with proven sepsis, bacterial cultures were positive for Staphylococcus aureus in 7/14 (50%), coagulase-negative Staphylococcus in 3/14 (22%), beta-hemolytic Streptococcus in 1/14 (7%), Klebsiella pneumonia in 1/14 (7%), Escherichia coli in 1/14 (7%) and Bacillus cereus in 1/14 (7%). This last patient developed a Bacillus cereus sepsis with brain abscesses after an ET performed through an umbilical venous catheter. This exceptional case has previously been reported.[23]

The rate of leukocytopenia was significantly higher in the ET-group than in the no-ET-group (88% versus 23%, OR 24.7, 95% CI 13.4-45.5). All ET-treated neonates with proven sepsis had leukocytopenia during admission and in 55% (6/11) leukocytopenia occurred after ET.

Umbilical venous catheterization was performed significantly more often in the ET-group than in the no-ET-group (99% versus 30%) (Table 1).

Hematological complications

The rate of thrombocytopenia (platelet count < 150×10^9/L) was significantly higher in the ET-group than in the no-ET-group (99% versus 32%, OR 143.8, 95% CI 34.6-598.6), and this was also true for severe thrombocytopenia and very severe thrombocytopenia (Table 2). Seventy-five of 134 ET-treated neonates (57%, 2 missing values) were treated with at least one platelet transfusion. One near-term neonate with Rhesus D alloimmunization born at 36 weeks' gestation received a platelet transfusion after ET on day one because of a post-ET platelet count of 39×10^9/L. On day 2 a cranial ultrasound showed a hemorrhage in the right parieto-occipital periventricular white matter. This hemorrhage was not seen on antenatal ultrasounds. Magnetic resonance imaging showed no signs of sinus thrombosis and coagulation was normal. At one year of age, the infant had no neurologic sequelae on physical examination. In the no-ET-group 3 neonates had signs of 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).

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

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

ET = exchange transfusion; Respiratory support = continuous positive airway pressure and/or mechanical ventilation; NEC = necrotizing enterocolitis; DIC = disseminated intravascular coagulation; NC = not calculated; OR = odds ratio; CI = confidence interval

Table 3 Complications during admission in Adjusted ET-group and no-ET-group

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

[a] Only complications which occurred after first ET were analyzed
[b] Assessed in 129/134 neonates

Neonatal mortality

Only one of the 347 neonates died during admission. This neonate from the no-ET-group, born at 30 weeks' gestation, suffered from severe fetal hydrops and died on day 11 due to multiple organ failure including respiratory failure due to respiratory distress syndrome and severe pulmonary hypertension, bilateral intraventricular hemorrhage grade 2 and renal failure.

ET guideline change

To measure the possible effect of ET guideline change[12] on the rate of the previously mentioned complications (Table 2) we performed a sub-analysis of ET treated neonates before and after guideline change. The rates of complications were not significantly different between both groups (data not shown).

Multivariate logistic regression analysis

Complications during entire admission

On multivariate logistic regression analysis 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 the significant differences at baseline (Table 1) and for the latter because preterm neonates are more susceptible for ET-related complications.[10,24] Since the presence of an umbilical venous catheter and ET are correlated (Spearman correlation coefficient $r = 0.680$, $p = <0.001$), we did not correct for the presence of an umbilical venous catheter. Multivariate regression analysis demonstrated that four complications, i.e. proven sepsis (OR 8.3, 95% CI 1.7-40.3), severe thrombocytopenia (OR 31.4, 95% CI 14.0-70.4), leukocytopenia (OR 36.0, 95% CI 17.5-73.8) and hypocalcemia (OR 27.4, 95% CI 5.9-126.8), had a higher incidence in the ET-group than in the no-ET-group (Table 2). Because none of the neonates in the no-ET-group had hypernatremia, additional multivariate logistic regression analyses could not be performed.

Complications after first ET

In Table 2 all complications during entire admission in both groups are reported. 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). Results are shown in Table 3.

Discussion

This study demonstrates that treatment with ET in neonates with HDN 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.

In the last three decades several studies have been published on neonatal morbidity due to treatment with ET.[4-11] 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%.[6,8,10] 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 than in the no-ET-group. The exact cause of the increased risk of infection is not fully understood, but is most probably related to the use of umbilical lines for ET. Umbilical catheters are a well known risk factor for nosocomial infection.[25] 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.[7,26] 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.[19,26-28] Finally, limited data on leukocytopenia in neonates with HDN due to red cell alloimmunization show that the risk of leukocytopenia is increased in severe Rhesus HDN.[29-32] 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 reported risk associated with ET is thrombocytopenia due to the use of a blood product which consists of thrombocyte-free erythrocytes.[18,19,33] Previous studies reported incidences of ET-related thrombocytopenia ranging from 6% to 44%.[4-6,8,10,11] In our study, the incidence of thrombocytopenia ($< 150\times10^9$/L) in the ET-group was much higher, almost all neonates (99%) had low platelet counts and 63% had severe thrombocytopenia (platelets $< 50\times10^9$/L). The differences in incidence can be explained by several factors including methodological differences and differences in study population. We only included neonates with red cell alloimmune HDN which is known to be associated with an increased risk for thrombocytopenia, even without ET.[16,34-36] 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 group treated with ET. Neonatologists must be aware of this potentially devastating complication as massive hemorrhage may arise after ET. Complications can be prevented by prophylactic platelet

transfusion before, during or after ET in case of low platelet counts. In our study, platelet transfusions were administered in 57% of neonates before, during or after ET.

A third known complication of ET is hypocalcemia, resulting from the use of citrated blood which contains almost no free calcium.[18,19,33,37] Previous studies reported incidences of hypocalcemia ranging from 3% to 42%.[4,8,10,11] In our study, 22% of neonates in the ET-group had a serum calcium < 8 mg/dL, and 13% of all hypocalcemic infants needed replacement therapy. We found that 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 which appears to be related to ET is hypernatremia. Hypernatremia probably results from an increased level of sodium in citrated blood (in our center 168 mEq/L). Only few studies have reported hypernatremia resulting from ET, and the exact incidence is unknown.[38,39] 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%.[4,8-10] In accordance with our findings, three recent studies reported no neonatal deaths after ET.[5,6,11] However, all these studies (as well as ours) were not powered to detect a difference in neonatal mortality. Differences between the reported rates can be explained by methodological differences such as different sizes of the study cohorts and differences in disease-severity between the cohorts. Neonates included in previous studies were often more premature than the near term-age population in our cohort.[9-11] Another explanation could be that our center is the national referral center for intrauterine treatment of red cell alloimmunization. Consequently nearly all severely affected neonates with HDN due to red cell alloimmunization are born and treated in our center. As a result, ET is a frequently performed and standardized procedure in our unit and part of routine practice. We speculate that this may have contributed to the low level of severe morbidity or mortality in the ET-group.

To our knowledge this is the first large study comparing neonatal complications in a group of infants with red cell alloimmunization treated with ET and a control-group without ET. Nevertheless, the results of this study should be interpreted with care because of the retrospective study design.

In conclusion, sepsis, leukocytopenia, thrombocytopenia, hypernatremia and hypocalcemia are common complications in neonates with HDN 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.

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6

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

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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.

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.

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-

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
HDN = hemolytic disease of the newborn; IUT = intrauterine transfusion

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 - n[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 - n[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] OR = odds ratio; CI = confidence interval; IUT = intrauterine red cell transfusion

29.26, p=0.009), treatment with IUT (OR 7.84, 95% CI 2.36-26.05, p<0.001), 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 transferase (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 Rh D 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 has 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.

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Thrombocytopenia at birth in neonates with red cell alloimmune hemolytic disease

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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 $<150x10^9$/L) and severe thrombocytopenia (platelet count $<50x10^9$/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 <150x10^9/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.

Materials 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 <150x10^9/L and was classified as mild (101 to 149x10^9/L), moderate (51 to 100x10^9/L), severe (21 to 50x10^9/L) and very severe (≤20x10^9/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 20x10^9/kg was given at birth if: (1) platelet count was <20x10^9/L (before November 2009 <30x10^9/L) in clinically stable neonates; (2) platelet count was <50x10^9/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 <10th 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.

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 (delivered at 30 weeks' gestation) with intraventricular hemorrhage grade 2 on day one. His platelet count at birth was $53x10^9$/L.

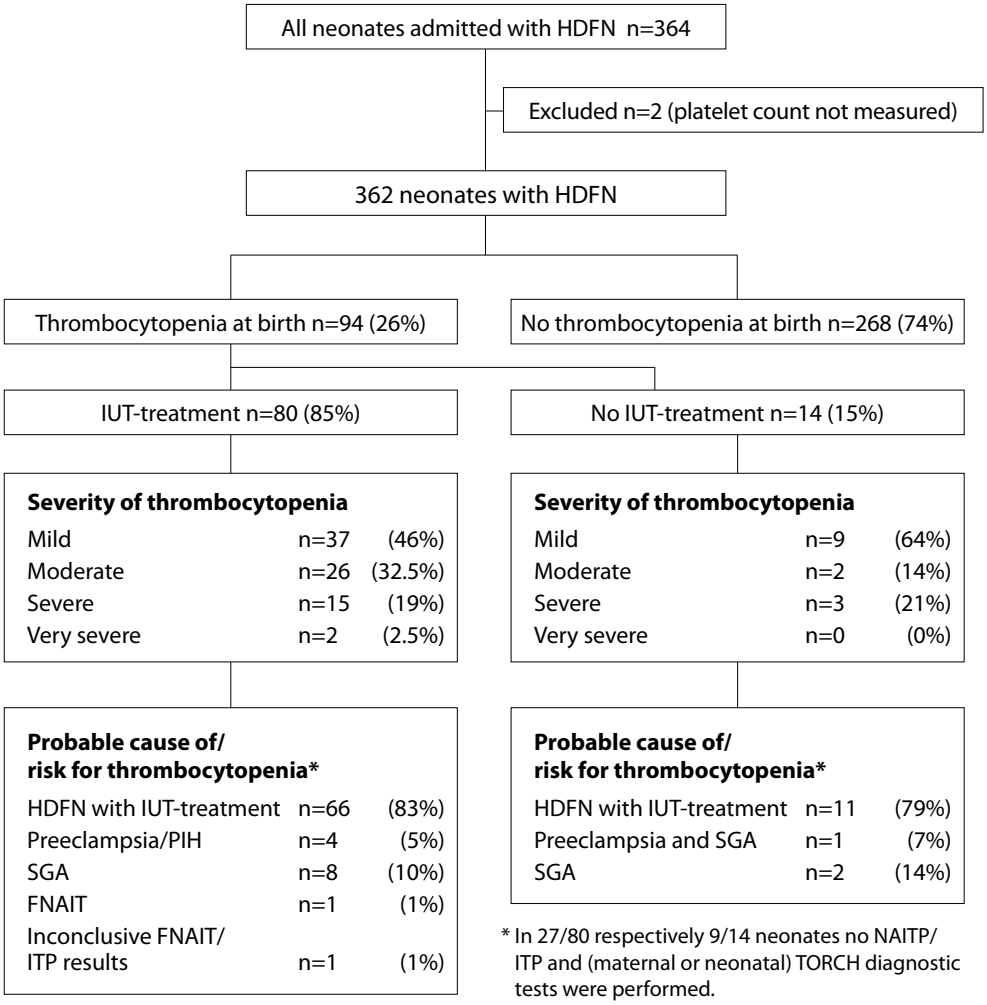
Four percent (14/362) of neonates received a platelet transfusion at birth due to thrombocytopenia.

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] = median (range), * based on all fetal platelet counts before each IUT, [b] = mean ± SD, HDFN = hemolytic disease of the fetus/newborn; IUT = intrauterine transfusion; SGA = small for gestational age

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



* In 27/80 respectively 9/14 neonates no NAITP/ ITP and (maternal or neonatal) TORCH diagnostic tests were performed.

HDFN = hemolytic disease of the fetus/neonate; IUT = intrauterine transfusion; PIH = pregnancy-induced hypertension; SGA = small for gestational age; FNAIT = fetal/neonatal alloimmune thrombocytopenia; ITP = immune thrombocytopenic purpura

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 $27x10^9$/L in addition to a single IUT at 33 weeks' gestation. He was born after 35+4 weeks' gestation with a birth weight of 2580 g (p25-p50) and Apgar scores of 4, 7 and 7 after 1, 5 and 10 min, respectively. Platelet count at birth was $22x10^9$/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 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 $\leq 50x10^9$/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

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] Median (IQR), [b] 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, HDFN = hemolytic disease of the fetus/newborn; PIH = pregnancy induced hypertension; IUT = intrauterine transfusion; SGA = small for gestational age

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 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 sings 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, 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.

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8

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

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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.

Materials 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),[2] (early criterium) 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 criterium 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 and criteria for top-up transfusion were the same in both groups. Neonatal anemia, often referred to

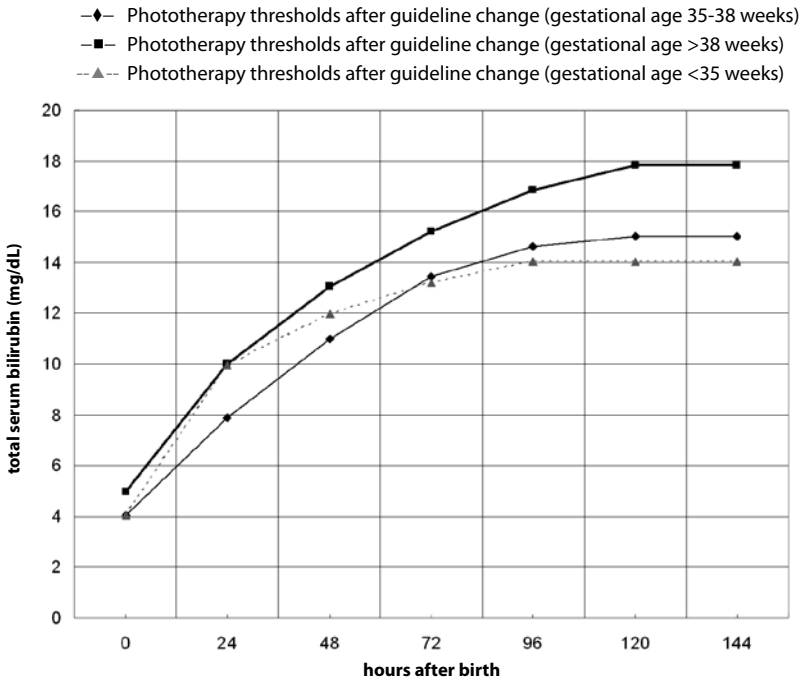

Figure 1 Phototherapy thresholds before and after guideline change in neonates with Rh hemolytic disease

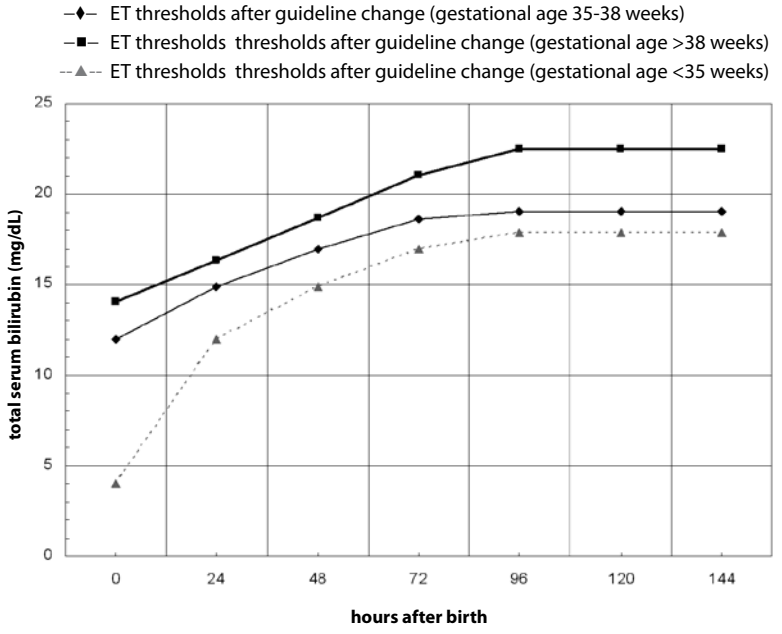

Figure 2 ET thresholds before and after guideline change in neonates with Rh hemolytic disease

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 delivered ≥ 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) ($p = 0.01$).

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]Reticulocyte count at birth was 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

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.

Comment

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.

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9

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

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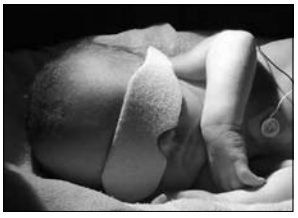

Abstract

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.

Materials and Methods

All (near)-term neonates (gestational age ≥ 35 weeks) with hemolytic disease due to maternal Kell and Rh D alloimmunization, delivered 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 delivered 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.

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 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] Reticulocyte count at birth was assessed in 15/34 and 81/157 neonates in the Kell and Rh D-group, respectively
[d] Days of phototherapy was assessed in 134/157 neonates with Rh D

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

Comment

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 (hemoglobinised) 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 colleges found no correlation between the antibody titer and the degree of inhibition of Kell-positive erythroid progenitor cells. 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]

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10

Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study

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Abstract

Objective: To determine the incidence and risk factors for neurodevelopmental impairment (NDI) in children with hemolytic disease of the fetus/newborn treated with intrauterine transfusion (IUT).

Study Design : Neurodevelopmental outcome in children at least 2 years of age was assessed using standardized tests, including the Bayley Scales of Infant Development, the Wechsler Preschool and Primary Scale of Intelligence and the Wechsler Intelligence Scale for Children, according to the children's age. Primary outcome was the incidence of NDI defined as at least one of the following: cerebral palsy, severe developmental delay, bilateral deafness and/or blindness.

Results: A total of 291 children were evaluated at a median age of 8.2 years (range 2 to 17 years). Cerebral palsy was detected in six (2.1%) children, severe developmental delay in nine (3.1%) children and bilateral deafness in three (1.0%) children. The overall incidence of NDI was 4.8% (14/291). In a multivariate regression analysis including only pre-operative risk factors, severe hydrops was independently associated with NDI (OR 11.2, 95%, CI 1.7-92.7).

Conclusions: Incidence of NDI in children treated with IUT for fetal alloimmune anemia is low (4.8%). Prevention of fetal hydrops, the strongest pre-operative predictor for impaired neurodevelopment, by timely detection, referral and treatment may improve long-term outcome.

Introduction

Fetal and neonatal hemolytic disease results from maternal alloimmunization to red cell antigens, for which mother and fetus are incompatible. Maternal IgG antibodies pass the placenta into the fetal circulation and cause destruction of fetal red cells. The resulting progressive fetal anemia leads, when untreated, to fetal hydrops and perinatal death.[1]

Before 1970, hemolytic disease due to antibodies against the Rhesus-D antigen was the most important cause of perinatal death.[2] Several interventions have drastically reduced the incidence and severity of the disease, including postnatal and more recently antenatal anti-D prophylaxis programs,[3,4] improved diagnostic management and neonatal treatment.[1,5-7] One of the major advances was the introduction in 1963 of intrauterine blood transfusions (IUTs),[1] first performed by Liley using the intraperitoneal technique.[8] In the 1980's, this technique was replaced by the intravascular IUT.[1] Nowadays, this treatment is the most successful procedure in fetal therapy, with perinatal survival rates exceeding 95% in experienced centers.[1,7] However, one of the concerns of the more widespread and successful use of fetal therapy is that a decrease in perinatal mortality may lead to an increase of children with long-term handicaps. Only a few studies with small patient numbers have reported on long-term neurodevelopmental outcome after IUT, with an incidence of adverse outcome ranging from 4.5 to 12%.[9-16] The aim of our study was to determine the incidence and risk factors for adverse neurodevelopmental outcome after IUT treatment in the largest cohort of children worldwide.

Methods

In 2008 we designed a large national cohort study to evaluate the long-term neurodevelopmental outcome in children treated with IUT: the **LOTUS** study (**LO**ng-**T**erm follow-up after intra-**U**terine transfusion**S**).[17] All mothers with red cell alloimmunization treated with IUT between January 1st 1988 and January 1st 2008 at the Leiden University Medical Center and their children were invited to participate in this large follow-up study. For the purpose of this study we included all children of 2 to 17 years of age who had complete follow-up including a cognitive development test. Children with severe congenital anomalies and syndromal disorders were excluded. This study was approved by the ethics committee of the Leiden University Medical Center. Informed consent was obtained from all participating families. A limited outcome evaluation in a small part of our study group (11 children treated between 1991 and 1993) was described before.[9] Primary outcome was a composite outcome termed neurodevelopmental impairment (NDI) defined as at least one of the following; cerebral

palsy (CP), severe cognitive developmental delay (< –2 Standard Deviation (SD)), bilateral deafness requiring hearing amplification and/or bilateral blindness.

The Leiden University Medical Center serves as the single national reference center for the management of red cell alloimmunization in pregnancy in the Netherlands. IUTs are performed when signs of fetal anemia are detected on Doppler ultrasound examinations. Details on our management guidelines for alloimmunized pregnancies were previously described.[18] Since the implementation of the IUT program using the ultrasound-guided intravascular transfusion technique at our center in 1987, all relevant perinatal data have prospectively been collected in a computerized database. Data included are: type of alloimmunization, gestational age at IUT, hemoglobin level, presence and severity of hydrops at the start of the intrauterine treatment, number of IUTs, gestational age at birth, gender, birth weight and neonatal outcome. Neonatal outcome data included: number of exchange transfusions due to severe hyperbilirubinemia, respiratory distress syndrome, necrotizing enterocolitis (classified according to Bell[19]), sepsis (defined as clinical symptoms of infection and a positive bacterial blood culture) and severe cerebral injury detected either on cranial ultrasound, Computed Tomography scan (CT) or Magnetic Resonance Imaging (MRI). Severe cerebral injury was defined as the presence of intraventricular hemorrhage ≥ grade 3 (classified according to Volpe[20]), cystic periventricular leukomalacia ≥ grade 2 (classified according to de Vries[21]) and/or ventricular dilatation (defined according to Levene et al[22]). Other major cerebral abnormalities associated with adverse neurological outcome were also recorded and classified as severe cerebral lesions. We recorded the presence of perinatal asphyxia, defined as three or more of the following five criteria: non-reassuring cardiotocogram patterns, umbilical cord arterial pH < 7.10, Apgar score < 5 at 5 minutes after birth, failure of spontaneous breathing at 5 minutes after birth and onset of multiple organ failure.

Parental education was determined by the level of education of each parent individually. A score of 1 was given if the parent's education was low, a score of 2 for an average educational level, and a score of 3 for higher levels of education. Education scores of both parents were then added (score range from 2 to 6). Ethnicity was recorded as Caucasian or non-Caucasian. Children were considered to be Caucasian when one or both parent(s) were of Caucasian ethnicity.

Follow-up

All participating families visited our out-patients clinic from August 2008 to November 2010. At this visit, a physical and neurological examination according to Touwen[23] and an assessment of cognitive development using standardized tests were performed.[17] All children were assessed by one of the three investigators specialized in developmental assessment (IL, VS and EL).

Presence of CP was assessed according to the criteria of the European CP Network and classified as diplegia, hemiplegia, quadriplegia, dyskinetic or mixed.[24] Minor neurological dysfunction (MND) was defined as a moderate abnormality of tone, posture, and movement leading to only minor functional impairment or minor developmental delay.[23]

Cognitive development in children aged 2 to 3 years was assessed according to the Dutch version of the Bayley Scales of Infant Development, 2nd edition (BSID-II).[17] BSID-II scores provide a mental developmental index (MDI). Children between 3 and 7 years of age were tested with the Dutch version of the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III-NL).[17] Cognitive development in children between 7 and 17 years of age was assessed with the Dutch version of the Wechsler Intelligence Scale for Children, 3rd edition (WISC-III-NL).[17] Both the WPPSI and the WISC provide a full scale IQ score. BSID-MDI, WPPSI and WISC scores follow a normal distribution curve with a mean score of 100. A score of 70-84 indicates mild delay (i.e. < -1 SD) and a score < 70 indicates severe delay (i.e. < -2 SD). A trained psychologist (JK), blinded to the antenatal course and neonatal outcome, performed the tests in all children.

Risk factors

Potential risk factors for NDI were investigated including severity of fetal anemia (actual hemoglobin level and Z-hemoglobin), presence and severity of fetal hydrops (classified according to van Kamp et al.[25]) at start of the intrauterine treatment, number of IUTs, gestational age at birth (divided in three groups: neonates born before 32 weeks' gestation, between 32 and 35 weeks' gestation and after 35 weeks' gestation), severe neonatal morbidity and perinatal asphyxia. Standardized Z scores of hemoglobin (Z-hemoglobin) were defined as the number of standard deviations (SDs) that an actual value deviated from the normal mean for gestational age. Reference values for hemoglobin were derived from the literature.[26] Severe neonatal morbidity was defined as the presence of one or more of the following: respiratory distress syndrome, necrotizing enterocolitis ≥ grade 2, sepsis and/or severe cerebral injury.

Statistical analyses

We used univariate logistic regressions to test the association between NDI and the potential risk factors. We entered the risk factors into a multivariate logistic regression model and included additional potential confounders including gender, parental education and ethnicity. Multiple logistic regression analysis was used to measure the independent effect of the potential risk factors for NDI. Results of logistic regression were considered significant at p-values < 0.05. We used the Pearson correlation test to calculate the correlation between hemoglobin at first IUT and IQ score. Analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Results

During the study period 1284 IUTs were performed in 451 fetuses. Thirty-one fetuses died in utero and 11 in the neonatal period resulting in a perinatal survival rate of 91% (409/451). Two more children died during childhood due to causes unrelated to hemolytic disease of the fetus/newborn (one accidental infant death occurred due to incorrect construction of the bedframe and one infant death was due to acute cardiomyopathy and pulmonary hypertension). Thus, the overall survival rate was 90% (407/451). Three children were diagnosed with congenital anomalies including Kinsbourne's syndrome, congenital cerebellar hypoplasia and Phelan-McDermid syndrome and were excluded from further analysis. A total of 342 children were 2 to 17 years of age and thus eligible for the study. Fifty-one (15%) children were lost-to-follow-up, due to declined consent (6%, 21/342) or loss of contact address (9%, 30/342). Complete follow-up data were obtained from 291 children by a visit at our out-patient clinic. A flowchart showing the derivation of our study population is shown in Figure 1.

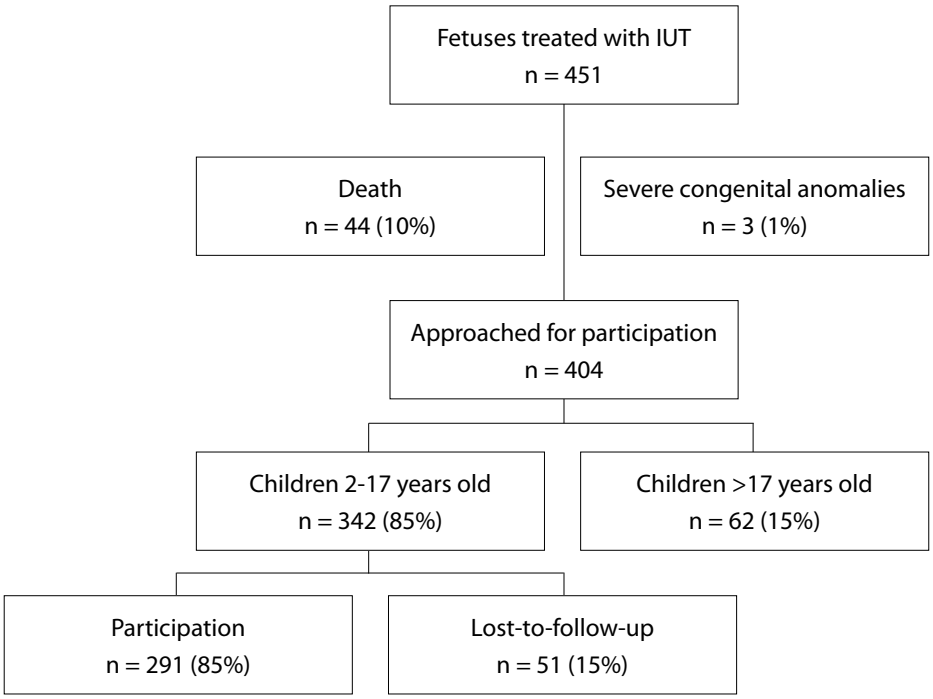


Figure 1 Flowchart showing the derivation of our study population

Perinatal outcome

Detailed information on the baseline perinatal characteristics on 291 long-term survivors is summarized in Table 1. The mean hemoglobin level at first IUT was 5.5 g/dL (± 2.4 SD), and the Z-hemoglobin -7.3 SDs. Both the mean hemoglobin level and Z-hemoglobin in fetuses with hydrops (mild or severe) were significantly lower than in fetuses without hydrops, 3.3 versus 6.3 g/dL ($p < 0.001$) and -9.1 versus -6.7 ($p < 0.001$).

The percentage of neonates born < 32 weeks', between 32 and 35 weeks', and ≥ 35 weeks' gestation was 2% (6/291), 15.5% (45/291) and 82.5% (240/291).

Exchange transfusions during the neonatal period were performed in 58% (168/291) of children. The following severe neonatal morbidities were recorded: respiratory distress syndrome (2.4%, 7/291), necrotizing enterocolitis (1.0%, 3/291), sepsis (5.8%, 17/291), perinatal asphyxia (3.8%, 11/291) and severe cerebral injury (1.7%, 5/291). Severe cerebral injury detected on cranial ultrasound included ventricular dilatation (n = 2), hemorrhagic periventricular leukomalacia (n = 1), cystic periventricular leukomalacia (n = 1) and extensive cerebral abscess (n = 1). In both children with ventricular dilatation, cerebral abnormalities were already detected antenatally. The incidence of severe neonatal morbidity was significantly higher in the group neonates born before 32 weeks' gestation (OR 32.1, 95% CI 5.4-190-8, $p < 0.001$). No significant differences in antenatal and neonatal characteristics were found between the follow-up (n = 291) and lost-to-follow-up group (n = 51).

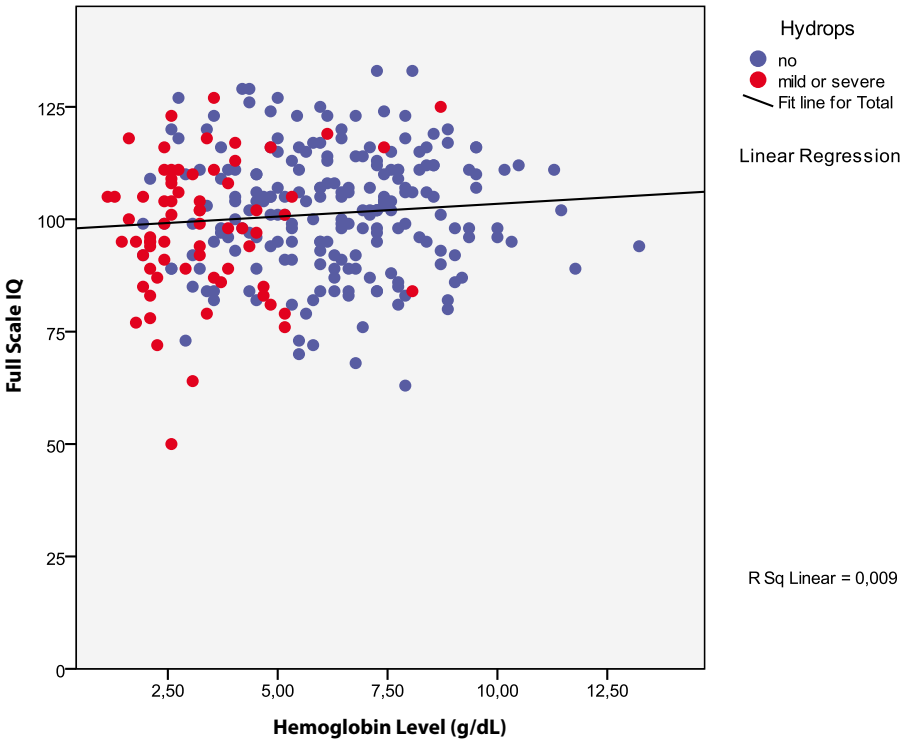
Table 1 Baseline characteristics

Rhesus D alloimmunization – n (%)	233 (80)
Kell – n (%)	36 (12)
Rhesus c – n (%)	15 (5)
Other – n (%)	6 (2)
Gestational age at first IUT[a] – weeks	26 ± 4.2 (16-35)
Number of IUTs per fetus[a]	3 ± 1.1 (1-6)
Hemoglobin at first IUT[a] – g/dL	5.5 ± 2.4 (1.1-13.2)
Hydrops – n/N (%)	75/291 (26)
Mild hydrops – n/N (%)	54/75 (72)
Severe hydrops – n/N (%)	21/75 (28)
Gestational age at birth[b] – weeks	36 (35-37)
Birth weight[b] – grams	2812 (2520-3159)
Neonates requiring an exchange transfusion – n (%)	168 (58)

[a] Values are given in mean ± 1 SD (range)
[b] Value given in median and interquartile range

Long-term neurodevelopmental outcome

The median age at follow-up was 8.2 years (range 2-17 years). The incidence of CP was 2.1% (6/291) (spastic quadriplegia: n = 3, spastic diplegia: n = 2, dyskinetic: n = 1). MND was recorded in 11.0% (32/291). None of the children had kernicterus. Nineteen children were evaluated using BSID-II tests, the average MDI score was 93 ± 14. A total of 89 children were tested using the WPPSI and 183 were tested using the WISC. The average full scale IQ in the WPPSI-group and WISC-group was 100 ± 14.8 and 101 ± 13.5, respectively. We found no correlation between hemoglobin level at first IUT and full scale IQ score (r = 0.1, p = 0.1) (Figure 2). Severe developmental delay (< -2 SD) was detected in 3.1% (9/291) of children. Moderate developmental delay (< -1 SD) was detected in 14.4% (42/291) of children. Bilateral deafness was present in three children (1.0%). None of the children had bilateral blindness. Table 2 summarizes the long-term neurodevelopmental outcome.



Q, intelligence quotient IUT, intrauterine transfusions

Figure 2 Relation between hemoglobin level at first IUT and full scale IQ score, in children with (red dots) and without (blue dots) fetal hydrops

Table 2 Long-term neurodevelopmental outcome in 291 long-term survivors after intrauterine transfusions

Age at follow-up[b] – years	8.2 (2-17)
Isolated severe development delay – n (%)	5 (1.7)
Isolated cerebral palsy – n (%)	2 (0.7)
Isolated bilateral deafness – n (%)	3 (1.0)
Cerebral palsy and severe developmental delay – n (%)	4 (1.4)
Neurodevelopmental impairment[b] – n (%)	14 (4.8)

[a] Value given as median (range)
[b] Neurodevelopmental impairment is defined as at least one of the following: cerebral palsy, severe development delay (< –2 SD), bilateral deafness or blindness

Overall, the incidence of NDI (CP, severe developmental delay, deafness and/or blindness) was 4.8% (14/291). Details on the combinations of abnormal findings in the children with adverse outcome are presented in Table 3. One infant with CP (#14 in Table 3) had no cranial ultrasound examination in the neonatal period, but a MRI performed at 2 years of age showed signs of cerebral atrophia, suggestive for periventricular leukomalacia. One infant with severe cerebral injury detected on ultrasound and MRI (hemorrhagic periventricular leukomalacia) in the neonatal period had a favorable outcome. Another infant with extensive Bacillus Cereus cerebral abscess had also a favorable outcome and was previously reported.[27]

The incidence of NDI was significantly higher in children with a history of mild and severe hydrops. Mild hydrops was present in 36% (5/14) of children with NDI compared to 18% (49/277) of children without NDI (OR 4.3, 95% CI 1.2 – 15.3, p = 0.025). Severe hydrops was present in 29% (4/14) of children with NDI compared to 6% (17/277) of children without NDI (OR 9.9, 95% CI 2.4 – 40.5, p = 0.001).

The risk of NDI was significantly increased in the group of neonates born prematurely (gestational age at birth < 32 weeks) (OR 12.8, 95% CI 2.1-79.5, p = 0.006) (Table 4), but was not increased in the group of neonates born between 32 and 35 weeks (OR 1.8, 95% CI 0.5-7.0, p=0.38) and ≥ 35 weeks' gestation (OR 0.4, 95% CI 0.1-1.3, p = 0.08).

Univariate analysis of potential risk factors for NDI was performed (Table 4). Several risk factors were found to be associated with NDI, including fetal hydrops, hemoglobin level, number of IUTs, prematurity and severe neonatal morbidity.

We found no difference between the groups with and without NDI for gender 57% (8/14 male) versus 55% (151/277 male) (p = 0.85) and ethnicity (Caucasian) 14% (2/14) versus 6%

Table 3 Data of 14 long-term survivors after intrauterine transfusions for fetal alloimmune anemia with neurodevelopmental impairment

Case	Hydrops	Hemoglobin -- g/dL - (GA at IUT - wk)	Number of IUT	GA at birth (wk)	Birth weight (grams)	Severe neonatal morbidity	Age at follow-up (yrs)	Bilateral deafness	Cerebral palsy	Severe developmental delay
1	none	5.3 (33)	1	35	2580	sepsis, asphyxia	8	no	diplegia	no
2	none	7.9 (26)	2	29	1460	PVL II, NEC III B	12.5	no	quadriplegia	yes
3	mild	3.9 (28)	3	33	3100	None	10	yes	no	no
4	mild	3.2 (24)	3	30	1700	RDS	8	yes	no	no
5	none	5.6 (22)	4	35	3020	None	2	no	no	yes
6	none	5.5 (24)	4	36	2750	None	8	no	no	yes
7	severe	3.1 (26)	4	35	2526	None	15	no	no	yes
8	severe	2.4 (26)	4	35	2835	ventricular dilatation	13.5	no	diplegia	no
9	severe	4.2 (22)	4	35	2460	ventricular dilatation	9.5	no	quadriplegia	yes
10	mild	4.7 (26)	4	34	3200	sepsis	10	yes	no	no
11	mild	1.5 (19)	5	37	3310	none	5	no	no	yes
12	mild	2.6 (21)	5	34	1915	none	2	no	dyskinetic	yes
13	none	6.8 (21)	5	38	2800	none	4.5	no	no	yes
14	severe	1.9 (18)	5	36	3035	none	14	no	quadriplegia	yes

GA = gestational age; PVL = periventricular leukomalacia; NEC = necrotizing enterocolitis; RDS = respiratory distress syndrome; IUT = intrauterine transfusion

Table 4 Analysis of potential risk factors for neurodevelopmental impairment (NDI)

	NDI (n = 14)	No NDI (n = 277)	p-value univariate analysis	OR (95% CI) univariate analysis	p-value multivariate analysis[d]	OR (95% CI) multivariate analysis[d]
Hydrops – n (%)	9 (64)	66 (24)	0.002	5.8 (1.9-17.8)	0.11	3.3 (0.76-14.5)
Hemoglobin at first IUT[b] – g/dL	4.2 ± 1.9	5.6 ± 2.4	0.032	1.3 per g/dL decrease (1.0-1.7)	-	-
Z-hemoglobin (SDs)	-8.1	-7.3	0.13	1.3 per SD decrease (0.6-1.1)	-	-
Number of IUTs[a]	4 (1-5)	3 (1-6)	0.018	1.7 per IUT (1.1-2.5)	0.02	2.3 per IUT (1.1-4.6)
GA at birth < 32 weeks – n (%)	2 (14)	4 (1)	0.006	12.8 (2.1-79.5)	0.54	2.3 (0.17-31.1)
Perinatal asphyxia – n (%)	1 (7)	10 (4)	0.51	2.0 (0.2-17.1)	0.19	5.8 (0.4-81.3)
Severe neonatal morbidity[c] – n (%)	6 (43)	16 (6)	< 0.001	13.1 (4.0-42.4)	< 0.001	85.6 (9.7-755.3)

[a] Value given as median (range)

[b] Value given as mean ±SD. GA = gestational age; IUT = intrauterine transfusion; OR = odds ratio; SD = standard deviation

[c] Severe neonatal morbidity is defined as at least one of following: respiratory distress syndrome, intraventricular hemorrhage ≥ grade 3, periventricular leukomalacia ≥ grade 2, necrotizing enterocolitis ≥ grade 2 and sepsis.

[d] Including parental education as a possible confounder

(18/277) (p = 0.24). Mean parental education was significantly lower in the NDI group compared to the no-NDI group 3.2 ± 1.1 vs. 4.2 ± 1.4, respectively (p = 0.016). Post-hoc analysis showed no difference in the incidence of exchange transfusion between the group with (57%, 8/14) and without NDI (58%, 160/277) (p = 0.96).

Potential risk factors and the possible confounder parental education were entered in a multivariate logistic regression model to assess the independent association with NDI (Table 4). We excluded hemoglobin at first IUT from this multivariate analysis model, as this variable is strongly associated with the presence of hydrops and could possibly bias our results. In a multivariate regression analysis including prenatal and postnatal factors, the following risk factors were independently associated with NDI: number of performed IUTs (OR 2.3 per IUT, 95% CI 1.1-4.6, p = 0.02), severe neonatal morbidity (OR 85.6, 95% CI 9.7-755.3, p < 0.001) and parental education (OR 8.4, 95% CI 2.2-31.5, p = 0.002).

To determine the predictive role of prenatal risk factors, we entered the following factors in a separate multivariate regression model using only the following prenatal factors: mild hydrops, severe hydrops, level of hemoglobin at first IUT and number of IUTs. We found that only severe hydrops (OR 11.2, 95% CI 1.7-92.7, p = 0.011) was significantly independent associated with NDI.

Comment

This is the largest study to date on long-term neurodevelopmental outcome in children surviving a high-risk pregnancy thanks to invasive fetal therapy. The vast majority (over 95%) of children treated with IUT for severe fetal anemia had a normal neurodevelopmental outcome. The incidence of severe developmental delay (3.1%) was in line with the Dutch normative population (2.3%).[28] In addition, the incidence of bilateral deafness in the general population was similar to what we found in our cohort.[29] However, the rate of CP (2.1%) in our study was higher compared to the general population (0.7% at 32 to 36 weeks' gestation[30] and 0.2% at 37 weeks' gestation[31]).

A few small studies on the long-term neurodevelopmental outcome in children treated with IUT have been reported.[9-16] The two largest studies to date reveal higher incidences of NDI when compared to our results, 10% (7/69) and 8% (3/38) respectively[9,10] Differences in long-term outcome may be explained by methodological differences and heterogeneity between the studies.

Apart from the reassuring results valuable for counseling pregnant women with red cell alloimmunization, the importance of our analysis lies in the identification of potentially avoidable risk factors for adverse outcome. The current study shows a clear association with long-term impairment and the presence of hydrops and number of IUTs performed. Severe fetal hydrops was already known to be associated with increased perinatal mortality.[25] The underlying mechanism causing cerebral damage and long-term NDI in hydropic and severely anemic fetuses is not yet known. Cerebral lesions may result from hypoxic injury related to severe anemia. Since short- and long-term outcome appears to be better in non-hydropic fetuses, clinicians should try to prevent or reduce the development of hydrops in fetuses at risk for fetal anemia. Interestingly, the actual hemoglobin concentration was more strongly associated with NDI than the hemoglobin Z-score. This concurs with the concept that tissue oxygenation depends more closely to the number of circulating red cells then on deviation of the hemoglobin level from the mean for gestational age. Whether more timely detection and treatment of fetal anemia, and prevention of hydrops improves outcome, and what degree of anemia actually requires transfusion needs further study.

Another risk factor for NDI was severe neonatal morbidity. As shown in our results, both the incidence of severe neonatal morbidity and the incidence of NDI were associated with the severity of prematurity. Severe prematurity is a well-known risk factor for neonatal morbidity, cerebral injury and long-term adverse outcome.[32,33] We did not find a relation between NDI and exchange transfusions, which we interpret as confirmation of our relatively aggressive neonatal management protocol aimed at reducing the rate of severe hyperbilirubinemia. None of the children had kernicterus.

Finally, parental education was independently associated with NDI. Socioeconomic status (SES) and parental educational level are well known determinants of child cognitive development.[28,34-37] Both factors may influence child cognitive functioning for a variety of reasons, including reduced access to essential material resources (such as cognitively stimulating materials) and/or non-material resources (such as education, information and skills). Moreover, genetic conditions may account for up to 72% of the variance in intelligence.[38]
The two most important limitations of our study were the relatively incomplete follow-up and the lack of a control group. We were not able to trace 9% of children, mainly due to the long time-lap since IUT treatment. In addition, 6% of families declined to participate to the study. The risk for an adverse outcome has been shown to be higher in the lost-to-follow group as children at increased risk for severe neurodevelopmental compromise may not return for evaluation.[39] Nevertheless, comparisons of antenatal and perinatal characteristics between the study group and the lost-to-follow-up showed no significant differences, suggesting that this type of bias was limited.

Conclusions

The high rate of intact survival in this high-risk group of severely anemic fetuses confirms the success of this antenatal treatment. Although hemolytic disease of the fetus/newborn was the main cause of perinatal death for many years, the chance of successful recovery with adequate antenatal management can nowadays be considered as excellent. However, several factors were associated with increased risk for NDI including fetal hydrops, number of IUTs and severe neonatal morbidity. Future studies to reduce the incidence of these risk factors in children treated with IUT may help decrease the rate of adverse long-term outcome.

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General discussion and future perspectives

Discussion

Fetal and neonatal red cell alloimmune hemolytic disease results from alloimmunization to red cell antigens, for which mother and fetus are incompatible. Production of maternal IgG antibodies directed against the fetal red blood cells occurs when fetal red blood cells positive for a certain antigen, pass into the blood circulation of a mother lacking that particular antigen. These maternal IgG antibodies may then cross the placenta into the fetal circulation and cause a wide scale of symptoms in the fetus, ranging from mild to severe hemolytic anemia and fetal hydrops.[1] Maternal immunization to the Rh D-antigen is the most common cause of severe fetal and neonatal disease.[2] However, more than 50 other (non-Rh D) red cell antigens have been reported to be associated with HDFN. Anti-Rh c and anti-Kell antibodies constitute the major causes of severe fetal and neonatal disease, whereas anti-Rh E, e, C, Cw and a few rare other antibodies are seen less frequently.[2-4] Non-Rh D-immunizations mostly result from incompatible red blood cell transfusions, if not precautionary measures e.g. Kell-matched transfusions for female in (pre)fertile age are applied.[2]

In the Netherlands, the post-delivery Rh D prophylaxis program (introduced in 1969) resulted in a decline of new Rh D immunizations from 3.5% in 1969 to 0.6% in 1995.[5] These rates were comparable with international studies on this subject.[6] In the Netherlands, around 170 pregnancies are affected each year with Rh D immunization and 380 with non-Rh D immunization. In approximately 30 of these cases severe fetal hemolytic disease will occur, requiring antenatal treatment with IUT in the LUMC.

In this chapter we summarize the recent evidence and opinions on management and outcome of HDFN due to red cell alloimmunization and discuss future research perspectives.

Management

HDN due to red cell alloimmunization can lead to severe hyperbilirubinemia, acute bilirubin encephalopathy and subsequently chronic bilirubin encephalopathy, also known as kernicterus.[7] Prevention of kernicterus is considered to be the primary goal of postnatal management of red cell alloimmune HDN.[8] Treatment of hyperbilirubinemia consists primary of intensive phototherapy and ET.[1] Phototherapy lowers serum bilirubin levels through photo-oxidation and converts bilirubin to a water-soluble substance.[7] Phototherapy was first introduced in the late 1950s, when white light was the mainstay of treatment.[9] Since then significant improvements have been made and it has become clear that the efficacy of phototherapy is dependent on a number of factors, including spectral quality of the delivered light, irradiance (intensity of light), surface area receiving phototherapy, distance from the light to the skin, skin pigmentation, total serum bilirubin concentration at the start of phototherapy and duration of expo-

sure.[1,7,10,11] In HDN due to red cell alloimmunization, prompt and intensive phototherapy should be started immediately after birth (as bilirubin can rise sharply after birth), in order to reduce the need for ET.[1] Intensive phototherapy implies the use of (1) emission of light in the blue-to-green range that overlaps the plasma bilirubin absorption spectrum (460-490 nm), (2) irradiance of at least 30 μW/cm^2/nm and (3) illumination of maximal body surface (diaper should be removed).[1,8,12] An exception on this intensive phototherapy regime is Kell alloimmunization, in which anemia is more prominent than hyperbilirubinemia. In Kell alloimmunization, anemia results mainly from reduced erythropoiesis by destruction of progenitor red blood cells rather than hemolysis of erythrocytes.[13] Consequently, only minimal phototherapy is required, despite severe anemia. Studies reporting on adverse effects of phototherapy are limited. In neonates with cholestasis, phototherapy can cause the bronze baby syndrome, in which skin, urine and serum evolve a greyish-brown discoloration.[7,14] The pathogenesis of this disorder is not fully understood, but it resolves spontaneously when phototherapy is discontinued. Recent reports from Swedish research groups have suggested an association between phototherapy and type 1 diabetes and childhood asthma.[15-17] The mechanism behind this association is unknown. However, effects of phototherapy on the neonatal gut and gut immune response have been suggested. Other studies have been reported on blue light phototherapy as a risk factor for melanocytic nevus development.[18,19] Additional prospective, multicenter studies are warranted to investigate the long-term adverse events of (intensive) phototherapy.

In case of failure of phototherapy, ET is used to remove bilirubin from the circulation. ET has the additional benefits of removing maternal antibodies (and consequently limiting further hemolysis) and correcting associated anemia.[1] Another favourable effect of ET is a decrease in plasma ferritin and iron levels.[20] ETs are performed with double volume transfusion (160 ml / kg) using irradiated, leucocyte-depleted compatible erythrocytes via an intravenous catheter, usually an umbilical vein. The rate of neonates with HDN requiring treatment with ET varies from 20 to more than 70%.[21] In 2004 more restrictive ET guidelines were published by the American Academy of Pediatrics[8] and which led to a decrease in the use of ET.[22] This reduction in ET has led to an increased need of top-up transfusions due to ongoing hemolysis and remaining antibodies.[22] Our center adopted the new guidelines in 2005 and the incidence of neonates with red cell alloimmunization requiring treatment with ET dropped significantly from almost 70% to less than 20% thereafter.[23]

After introduction in the late 1940s,[24-26] neonatal treatment with ET became one of the most frequently performed neonatal procedures. However, ET remains a procedure with a significant risk of adverse effects. The current mortality rate is reported to be less than 2%, whereas rates of morbidity and ET-related adverse events can reach 74%.[27-34] Reported

adverse events include mainly catheter-related complications (malposition, sepsis), complications linked to the use of blood products (thromboembolization, graft versus host reactions, infection), metabolic derangements (acidosis, disturbance of serum levels of sodium, calcium, potassium and glucose) and cardio-respiratory reactions (including cardiac arrhythmias, cardiac arrest and apnea).[27-34] Our study on morbidity after ET demonstrates that treatment with ET in neonates with HDN is associated with a 6-fold increased risk of sepsis (incidence 8% in the ET-group versus 1% in the no-ET-group, odds ratio (OR) 6.3, 95% confidence interval (CI) 1.7-22.9), a 25-fold increase in leukocytopenia (incidence 88% (versus 23%), OR 24.7, 95% CI 13.4-45.5), a 21-fold increase in severe thrombocytopenia (incidence 63% (versus 8%), OR 21.4, 95% CI 11.5-39.7), a 29-fold increase in hypocalcemia (incidence 22% (versus 1%), OR 29.1, 95% CI 6.8-124.5) and an increased risk of hypernatremia (incidence 8% (versus 0% in the no-ET-group)). Treatment with ET was not associated with neonatal death in our study population. The remarkably lower incidence of ET-related morbidity and mortality in our study compared to previous studies can be explained by methodological differences (different sizes of the various study cohorts) and differences in disease-severity between the studied cohorts (premature neonates in previous studies versus (near) term-age neonates in our cohort).[28,29,31] Another explanation could be that in the Netherlands treatment for intrauterine and postnatal red cell alloimmunization is centralized in one tertiary center. Subsequently almost all severely affected neonates with HDN due to red cell alloimmunization are born and treated in our center. As a result, ET is a frequently performed and standardized procedure in our unit and part of routine practise. We speculate that in experienced hands severe permanent sequelae due to ET-procedures can be kept at a minimum. We therefore advocate a centralized management of neonatal red cell alloimmunization.

Neonatal treatment with IVIg has been suggested as an alternative therapy for ET in HDN due to red cell alloimmunization.[8] In many Western countries, including the Netherlands, IVIg is widely used.[35] In a few small RCTs, IVIg reduced the need for ET and duration of phototherapy in neonates with red cell alloimmunization.[36-39] However, these studies were restricted by several important methodological limitations.[23] In 2002 a Cochrane review concluded that further well-designed trials are needed before routine use of IVIg can be recommended.[40] In the last decade, two other study-groups performed a RCT on this topic, favouring the use of IVIg. However, these studies were flawed due to with important methodological restrictions related to unclear randomization and blinding procedures.[41,42] In contrast, our double-blind, placebo-controlled RCT on the prophylactic use of IVIg in neonatal red cell alloimmunization demonstrated that IVIg does not reduce the need for ET nor the rates of other adverse neonatal outcomes.[23] Recently, a research group from Brazil finalized a similar RCT and also found no difference between both groups on the rate of ET.[43] A possible explanation for the

lack of effect of IVIg in our study could be that treatment with intensive and prophylactic phototherapy, starting immediately after birth, reduces the risk of severe hyperbilirubinemia.[23] In view of the absence of beneficial effects and because of rare but potential adverse effects,[44-46] we do not recommend the use of IVIg in HDN due to red cell alloimmunization.[23] A new meta-analysis of all recently published RCTs is needed to determine the efficacy and safety of IVIg in neonatal red cell alloimmunization.

In the past, various other treatment strategies for hyperbilirubinemia in neonatal red cell alloimmunization have been investigated, including treatment with albumin, phenobarbital, metalloporphyrines and clofibrate.[47] Administration of *albumin* before ET might increase the efficacy of ET, because more bilirubin will be mobilized and excreted from tissue to blood.[1,48] In 2009 Shahian et al. performed a RCT to determine the role of administration of intravenous albumin prior to ET in term, otherwise healthy neonates. They observed that infusion of 20% albumin one hour prior to ET significantly reduced the post-ET total serum bilirubin level and duration of phototherapy.[48] However, evidence that albumin infusion increases long-term outcome in infants with red cell alloimmunization is not available and thus routine use of albumin is not recommended.[1] *Phenobarbital* increases bilirubin uptake, conjugation and excretion[1] and its potential effect on hyperbilirubinemia has been studied for decades.[49-53] A recent retrospective study by Trevett et al. showed that antenatal maternal administration of phenobarbital significantly reduces the need for ET in neonates affected with HDN due to red cell alloimmunization. The incidence of ET in neonates with and without antenatal phenobarbital administration was 9% versus 52%, respectively ($p < 0.01$).[54] Further study in a randomized controlled trial is necessary to confirm these results. Recentlt, Chawla et al. performed a meta-analysis to evaluate the role of phenobarbital in the management of unconjugated hyperbilirubinemia during the first two weeks of life in preterm neonates. The authors reported that phenobarbital reduces peak serum bilirubin, duration and need of phototherapy and need of ET in preterm very low birth weight neonates. These impressive findings warrant further studies to evaluate adverse effects and neurodevelopmental outcome.[55] *Metalloporphyrins,* synthetic heme analogs, are competitive inhibitors of heme oxygenase, the rate-limiting enzyme in bilirubin production.[1,56] Their use has been proposed as an alternative strategy for treating severe hyperbilirubinemia by preventing the formation of bilirubin.[56] However, a recent Cochrane review suggests that placebo-controlled RCTs are required to report on outcomes such as severe hyperbilirubinemia, kernicterus, ET and long-term neurodevelopmental impairment.[52,56-58] Finally, a few studies report beneficial effects of *clofibrate* on hyperbilirubinemia. This drug activates peroxisome proliferator-activated receptors and increases bilirubin conjugation and excretion. One single dose of clofibrate has been reported to be effective, safe and cost-effective in view of reducing duration of admission.[59] However, long-term clofibrate treatment has

been associated with serious adverse effects and therefore more research is needed to clarify its safety.[47]

Short-term outcome

In the past, various postnatal complications in neonatal red cell alloimmunization have been reported, including hematological complications (anemia, thrombocytopenia and leucopenia) and cholestatic liver disease.[1,20]

Anemia

Anemia in red cell alloimmunization results from hemolysis of fetal red blood cells by maternal IgG antibodies. Maternal antibodies usually persist in the infants circulation for several months after birth, causing prolonged hemolysis. Anemia in HDN due to red cell alloimmunization can be divided into early onset anemia (within 7 days after birth), caused by antibody dependent hemolysis of red blood cells, and late onset anemia (from 1 week until 3 months after birth). Late onset anemia may be secondary to either ineffective erythropoiesis due to suppressed bone marrow ('late hyporegenerative anemia') and/or persistent hemolysis ('late anemia of hemolytic disease').[20,60] Late anemia is a common problem in infants with red cell alloimmunization and we therefore advocate that a full work-up, including invasive diagnostic tests to exclude other causes of anemia is generally not necessary. Treatment of anemia exists of top-up transfusions, which can be necessary up to the third month of life. Infants must therefore be checked for the rate of hemoglobin fall once a week until three months of age. Approximately 80% of infants treated with IUT require at least one top-up transfusion for late anemia in the first three months of life, compared to around 65% of infants without IUT.[1,22,60] International guidelines for top-up transfusions in the first months of life including transfusion triggers are not available. In our center transfusion triggers include: haemoglobin level < 8 g/dL (5 mmol/L) or < 9.6 g/dL (6 mmol/L) when clinical symptoms of anemia are present (need of extra oxygen, poor feeding, tachycardia, tachypnea).[1,20] Generally, top-up transfusions given to neonates with HDN consist of 10-20 mL/kg irradiated, ABO/Rh type-specific and antigen-negative red blood cells.[20]

Erythropoietin (EPO) can be used to prevent late anemia and reduces the need for top-up transfusions. However, there is insufficient evidence to recommend routine use of EPO in HDN due to red cell alloimmunization.[61-69] Larger RCTs are needed to study this topic.

Various supplements, including folic acid and iron could theoretically support erythropoiesis. However, evidence on optimal dosage and side effects is lacking. Nevertheless, in our center we routinely administer folic acid 0.05 mg/day orally to infants with HDN during the first three months of life.[1,20] Iron supplementation is sporadically used to support erythropoiesis in

anemic neonates with HDN. However, the vast majority of neonates usually do not lack iron, due to multiple intrauterine and/or postnatal transfusions. With each top-up transfusion, iron is transfused as well. Iron overload can cause damage to the liver, heart and other organs.[70] Therefore iron supplementation should be withheld, especially in transfused infants. More studies are needed to define indications for chelation therapy, which is sporadically used to treat iron overload in HDN.[71]

Thrombocytopenia

Limited studies have shown that fetuses with red cell alloimmunization are at increased risk of thrombocytopenia.[72-74] We investigated this topic in our study population and found that 26% of neonates with red cell alloimmunization had thrombocytopenia ($<150 \times 10^9$ / L) at birth.[75] Thrombocytopenia at birth was independently associated with IUT treatment, small for date and lower gestational age at birth. Etiologic factors contributing to thrombocytopenia in red cell alloimmunization include decreased production, increased destruction and dilution.[20] Moreover, thrombocytopenia is a well known complication of ET due to platelet-poor blood and/or catheter-related thrombosis.[76-78]

Leucopenia

Only scarce data are available on leucopenia in neonatal red cell alloimmunization. It appears that the incidence of neutropenia increases if HDN is more severe, but little is known about incidence and morbidity this complication deserves further scrunity.[79,80]

Liver disease

Cholestatic liver disease may occur in HDN due to red cell alloimmunization and has been associated with iron overload due to multiple IUTs.[81-84] However, data on incidence and severity is limited and little is known about pathogenesis, risk factors, neonatal management and outcome. In our study on this topic we found that cholestasis occurs in 13% of neonates with red cell alloimmunization and is independently associated with IUT treatment and Rh D type of alloimmunization. Extensive investigations were performed to rule out other causes of cholestasis, but all tests were normal. Cholestasis resolved spontaneously within 1 week to 3 months after birth in almost half of the patients. One patient was treated with iron chelation therapy due to a prolonged and severe course of hyperferritinemia. We suggest that a full work-up to exclude other causes of cholestasis in a child with red cell alloimmunization 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.

Long-term outcome

Before the LOTUS study, only a few small studies have reported on the long-term neurodevelopmental outcome after IUT with incidences of adverse outcome ranging from 4.5 to 12%.[85-92] In the LOTUS study, a large national cohort study designed to evaluate long-term neurodevelopmental outcome in children treated with IUT for red cell alloimmunization at our center, we examined 291 children at a median age of 8.2 years. The overall incidence of neurodevelopmental impairment (NDI) was low, 4.8% (including cerebral palsy, severe developmental delay and bilateral deafness). Several factors were associated with increased risk for NDI, including fetal hydrops, number of IUTs and severe neonatal morbidity. The high rate of intact survival proves the success of antenatal IUT-treatment. Whether reducing the incidence of risk factors (in particular severe hydrops) will also reduce the incidence of long-term neurodevelopmental outcome needs to be investigated in future studies.

Future perspectives

During the last decades, a significant evolution in prenatal and postnatal care strategies for patients with red cell alloimmunization has occurred. New management options have led to a remarkable decrease in perinatal mortality and morbidity. However, several questions are still unanswered. This paragraph focuses on future research perspectives.

Neonatal management

- Prospective, multicenter trials are warranted to investigate the long-term adverse events of (intensive) phototherapy, including bronze baby syndrome, asthma, type 1 diabetes and melanocytic nevus development.
- Prospective, double-blinded RCTs are necessary to evaluate benefits, adverse effects and neurodevelopmental outcome of administration of albumin, phenobarbital, metalloporphyrines and clofibrate in neonatal red cell alloimmunization.
- A new meta-analysis of all recently published RCTs on IVIg is needed to definitively establish the efficacy and safety of IVIg in neonatal red cell alloimmunization.
- Larger well-designed trials are needed to recommend on the use of EPO in neonates with HDFN to reduce the number of top-up transfusions.
- Studies on the use of folic acid are needed to determine if and in which dosage this therapy could be beneficial (although this is of minor importance compared to the issues mentioned here above).

Short-term outcome

- More studies are needed to determine the incidence and risk factors of iron overload in infants with HDN treated with and without IUT and to define indications for chelation therapy in infants with red cell alloimmune hemolytic disease.
- Large prospective follow-up studies are required to determine the exact course of cholestasis in neonates with red cell alloimmune hemolytic disease.
- Further research is required to study the prevalence and clinical significance of neutropenia and thrombocytopenia in relation to red cell alloimmunization and ET.

Long-term outcome

- Further studies are warranted to reduce the incidence of risk factors, including fetal hydrops, associated with adverse long-term outcome in children treated with IUT.
- More research is required to determine the effect of factors such as phototherapy, ET, IVIg and iron overload on the immune system and the risk of diabetes, allergy and asthma.

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12

Summary

Rhesus hemolytic disease of the neonate (RHDN) results from alloimmunization to red cell antigens, for which mother and fetus are incompatible. In RHDN, maternal immunoglobulin (IgG) antibodies cross the placenta and cause destruction of fetal red blood cells. RHDN may lead to excessive hyperbilirubinemia and prolonged fetal and neonatal anemia. Unconjugated bilirubin may pass through the blood-brain-barrier and lead to permanent brain damage due to kernicterus. Traditional neonatal treatment of RHDN consists of intensive phototherapy and exchange transfusion (ET).

In this thesis, several studies on neonatal red cell alloimmune hemolytic disease are presented, including various management options, associated complications and co-morbidities and the short-term and long-term outcome of children with RHDN.

In **Chapter 2** an overview of the literature is presented. This review focuses on the management of neonatal and pediatric complications associated with Rhesus hemolytic disease, discusses postnatal treatment options and summarizes the results of studies on short-term and long-term outcome.

In **Chapter 3** we present the results of a randomized double-blind, placebo-controlled trial, to test whether the prophylactic use of IVIg reduces the need for ETs in neonates with Rhesus hemolytic disease (HDN): the LIVIN study. ET is an invasive, high-risk procedure associated with a significant rate of adverse effects. To avoid ET, international guidelines recommend the use of intravenous immunoglobulin (IVIg) in neonates with Rhesus hemolytic disease in case of failure of phototherapy. However, recommendations for the routine use of IVIg are controversial because of the small number of RCTs reported on this topic and the methodological limitations of these studies. In the LIVIN study we found no difference in the rate of ETs between the IVIg and placebo groups (17% versus 15%), nor in duration of phototherapy (4.7 versus 5.1 days), maximum bilirubin levels (14.8 versus 14.1 mg/dL) and proportion of neonates requiring top-up red cell transfusions in the first three months of life (83% versus 87%). Our findings do not support the use of 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.

In **Chapter 4** we report a term neonate with RHDN treated with an ET through an umbilical venous catheter who developed brain abscesses due to a Bacillus cereus sepsis. This severe complication has not previously been reported. We discuss possible causes for this severe infection, discuss the possible association with ET treatment and provide suggestions for prevention.

In **Chapter 5** we present the results of a study on complications related to ET. As previously stated, ET is a high-risk invasive procedure requiring the use of central lines. Reported ET-related adverse events include mainly catheter-related complications, metabolic derangements, hematologic complications and cardio-respiratory reactions. To investigate morbidity and mortality rates associated with ET in our unit, we studied a large series of neonates with RHDN admitted to our center. We recorded the number and rate of complications during admission in the group of neonates treated with and without ET. A total of 347 infants with red cell alloimmune hemolytic disease were included, 39% was treated with at least one ET during admission (ET-group) and 61% did not require ET (no-ET-group). Comparison between the ET-group and no-ET-group showed that ET treatment was independently associated with: proven sepsis (8% versus 1% respectively), leukocytopenia (88% versus 23%), severe thrombocytopenia (platelet count < 50 x 10^9/L) (63% versus 8%), hypocalcemia (22% versus 1%) and hypernatremia (8% versus 0%). Neonatal death did not occur in the group treated with ET. We conclude that in experienced hands severe permanent morbidity and mortality rates due to ET-procedures can be reduced to a minimum.

Chapter 6 focuses on cholestasis, a frequently observed neonatal disorder associated with red cell alloimmunization. Etiology of cholestatic liver disease in neonates with RHDN has been associated with iron overload due to (multiple) IUT(s). Data on the incidence and severity of cholestasis in neonates with HDN due to red cell alloimmunization is scarce, and little is known about pathogenesis, risk factors, neonatal management and outcome. We retrospectively studied a large group of 313 infants with red cell alloimmune hemolytic disease treated with or without IUT, admitted to our center. We found that cholestasis occurred in 13% of these infants and was indepently associated with IUT treatment and Rhesus D type of alloimmunization. Although cholestasis is mild and transient in most cases, a few neonates have severe cholestatic liver disease with protracted course and require intensive treatment and in one case chelation therapy was needed. We therefore conclude that larger follow-up studies are required to determine the exact course and etiology of cholestasis in infants with red cell alloimmune hemolytic disease.

In **Chapter 7** we describe the occurrence of thrombocytopenia at birth, another frequently noticed disorder associated with RHDN. Limited studies have shown that fetuses with red cell alloimmunization are at increased risk of thrombocytopenia. However, incidence and severity of and risk factors for thrombocytopenia at birth in neonates with red cell alloimmunization is unclear. Therefore we retrospectively investigated the platelet count at birth in 362 neonates with red cell alloimmunization admitted to our center. We determined the incidence of thrombocytopenia (platelet count < 150x10^9/L) and severe thrombocytopenia (platelet count < 50x10^9/L) and evaluated risk factors for thrombocytopenia. We found that

thrombocytopenia was present in 26% of included neonates at birth. Severe thrombocytopenia was found in 6% of neonates. Only one neonate with thrombocytopenia had clinical sings of bleeding at birth (intraventricular hemorrhage grade 2). Although this neonate was thrombocytopenic at birth, other factors such as prematurity and hydrops could have contributed to this bleeding complication. We found that three risk factors were independently associated with thrombocytopenia at birth: treatment with IUT, small for gestational age (SGA) and lower gestational age at birth.

In **Chapter 8** we studied the effect of a restrictive guideline for ET on the number of top-up transfusions (red blood cell transfusions) in neonates with RHDN in the first three months of life. In December 2005 we changed our ET policy (according to the recommendations of the American Academy of Pediatrics) from using liberal ET criteria to more restrictive ET criteria. In this study we included 183 (near)-term neonates with RHDN admitted to our center. 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. The percentage of neonates requiring an ET decreased significantly from 66% in group I to 26% in group II. The percentage of neonates receiving a top-up transfusion increased from 68% in group I to 81% in group II. We conclude that restrictive ET criteria in neonates with RHDN lead to a reduction of the rate of ET but an increase in the number of top-up transfusions for neonatal anemia.

The aim of the study described in **Chapter 9** was to evaluate neonatal and hematological outcome in a large series of neonates with Kell HDN compared to neonates with Rhesus D HDN. Kell type of red cell alloimmunization is second only to Rhesus D in causing antibody-mediated fetal anemia and accounts for 10% of all antibody-mediated fetal anemias. In contrast to Rhesus D HDN, fetal anemia in Kell HDN is primarily due to concomitant suppression of erythropoiesis rather than hemolysis of erythrocytes and is thus associated with milder hyperbilirubinemia. Consequently, the immediate neonatal management in Kell HDN is different from Rhesus D HDN and is mainly based on top-up transfusions rather than phototherapy or ET. In this study, we included 191 neonates and found that fetuses with severe Kell HDN were more often treated with IUT than fetuses with Rhesus D HDN (82% versus 66% respectively). Infants with HDN due to Kell-antibodies needed less phototherapy (2.4 versus 4.1 days) and ETs (6% versus 62%) in the neonatal period than neonates with Rh D hemolytic disease. However, the need for top-up transfusions was similar in both groups (62% versus 72%), justifying similar follow-up management as in Rhesus D hemolytic disease.

The long-term neurodevelopmental outcome in children with alloimmune hemolytic disease of the fetus/newborn treated with IUT is presented in **Chapter 10**. Nowadays, treat-

ment with IUT is the most successful procedure in fetal therapy, with perinatal survival rates exceeding 95% in experienced centers. However, one of the concerns of the more widespread and successful use of fetal therapy is that a decrease in perinatal mortality may lead to an increase of children with long-term handicaps. Only a few studies with small patient numbers have reported on long-term neurodevelopmental outcome after IUT. Therefore the LOTUS study was designed. The aim of this study was to determine the incidence and risk factors for adverse neurodevelopmental outcome after IUT treatment in the largest cohort of children worldwide. Neurodevelopmental outcome in children at least 2 years of age was assessed using standardized tests, including the Bayley Scales of Infant Development, the Wechsler Preschool and Primary Scale of Intelligence and the Wechsler Intelligence Scale for Children, according to the children's age. Primary outcome was the incidence of neurodevelopmental impairment (NDI) defined as at least one of the following: cerebral palsy, severe developmental delay, bilateral deafness and/or blindness. A total of 291 children were evaluated at a median age of 8.2 years (range 2 to 17 years). Cerebral palsy was detected in 2.1% of children, severe developmental delay in 3.1% of children and bilateral deafness in 1.0% of children. The overall incidence of NDI was 4.8%. We also found that severe hydrops was independently associated with NDI. We concluded that prevention of fetal hydrops by timely detection, referral and treatment may further improve long-term outcome.

In conclusion, perinatal morbidity and mortality rates in red cell alloimmunization decreased remarkably during the last 50 years due to the significant evolution in prenatal and postnatal care strategies. However, several questions still remain unanswered and provide a basis for future research.

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Samenvatting

Tijdens de zwangerschap kan zogenaamde zwangerschapsimmunisatie ontstaan. Hierbij maakt de moeder antistoffen tegen een stof (bloedgroepantigeen) die niet op haar eigen rode bloedcellen (erytrocyten) aanwezig is, maar wel op die van de foetus. Als deze antistoffen van de moeder (zogenaamde immunoglobulinen type IgG) de placenta kunnen passeren en in de bloedsomloop van de foetus komen, kunnen ze het bloed van de foetus afbreken waardoor deze bloedarmoede (anemie) krijgt. De afbraak van erytrocyten wordt hemolyse genoemd en het ziektebeeld heet derhalve hemolytische ziekte van de foetus en pasgeborene (HZFP). Het sterkste bloedgroepantigeen is het Rhesus D-antigeen en om die reden wordt HZFP ook wel Rhesus (hemolytische) ziekte genoemd.

HZFP kan bij de foetus en de pasgeborene (neonaat) leiden tot ernstige bloedarmoede door sterk verhoogde bloedafbraak. Bij de afbraak van rode bloedcellen komt bilirubine vrij in de bloedbaan, een stof die een gele verkleuring van de huid geeft. Ongebonden (ongeconjugeerd) bilirubine kan bij pasgeborenen de bloed-hersen-barrière passeren,zich afzetten in het hersenweefsel en tot ernstige hersenschade leiden. Dit beeld wordt kernicterus genoemd. Het kind kan blijvende neurologische verschijnselen en gehoorverlies hieraan overhouden of zelfs hierdoor overlijden. Als HZFP adequaat behandeld wordt, kan hersenschade voorkomen worden. Na de geboorte bestaat de traditionele behandeling van HZFP uit intensieve fototherapie (lichttherapie) en wisseltransfusie. Bij fototherapie wordt het ongeconjugeerde bilirubine onder invloed van licht omgezet in een wateroplosbare stof die het lichaam kan verlaten. Fototherapie bij HZFP dient direct na de geboorte gestart te worden, omdat het bilirubinegehalte snel kan stijgen. Dit houdt in dat een zo groot mogelijk huidoppervlak door fototherapielampen beschenen moet worden: het kind ligt zonder kleding en luier op een biliblanket (lichtmatje) en wordt beschenen door maximaal 4 spots. Als ondanks intensieve fototherapie het bilirubinegehalte verder doorstijgt, wordt een wisseltransfusie verricht. Met een wisseltransfusie wordt het bloed van het kind in kleine porties gewisseld voor bloed van een donor. Meestal wordt gewisseld met een volume bloed dat tweemaal zo groot is als het circulerend bloedvolume van de baby. Dit gebeurt via een katheter die aangelegd wordt in de navelstrengader. Met behulp van een wisseltransfusie worden het bilirubine en de hemolytische antistoffen verwijderd en kan tevens een eventuele bloedarmoede gecorrigeerd worden.

In dit proefschrift worden verschillende studies over HZFP gepresenteerd, waaronder studies over verschillende (andere) behandelingsmogelijkheden, bijkomende complicaties en comorbiditeit en korte en lange termijn uitkomsten van kinderen met HZFP.

Hoofdstuk 2 bevat een samenvatting van de literatuur en een overzicht van de behandeling van neonatale en pediatrische complicaties die geassocieerd zijn met HZFP. Ook worden de verschillende behandelingsopties besproken en de resultaten van studies over korte en lange termijn uitkomst.

In **Hoofdstuk 3** presenteren we de resultaten van een gerandomiseerde, dubbel-blinde, placebo-gecontroleerde studie die we uitgevoerd hebben op de afdeling neonatologie van het LUMC naar het effect van het preventief geven van intraveneus immunoglobuline (IVIg) direct na de geboorte. Het doel van deze studie (LIVIN studie) was om te onderzoeken of het gebruik van IVIg de behoefte aan wisseltransfusies zou doen afnemen bij pasgeborenen met HZFP. Een wisseltransfusie is een invasieve, risicovolle procedure met een aanzienlijke kans op complicaties. Complicaties die kunnen optreden zijn met name gerelateerd aan problemen die kunnen ontstaan met een katheter in de navelstrengader, zoals infecties, stolsels en bloedingen. Om wisseltransfusies te voorkomen, wordt in internationale richtlijnen geadviseerd om IVIg te gebruiken bij pasgeborenen met HZFP als fototherapie faalt. Aanbevelingen voor het routinematig gebruik van IVIg zijn echter controversieel vanwege het kleine aantal studies dat bekend is over dit onderwerp en de methodologische beperkingen van deze studies. In de LIVIN studie vonden we geen verschil in het percentage wisseltransfusies tussen de IVIg- en de placebogroep (17% versus 15%), noch in de duur van de fototherapie (4,7 versus 5,1 dagen), maximum bilirubine (14,8 versus 14,1 mg/dL) en het percentage pasgeborenen dat een zogenaamde top-up transfusie met rode bloedcellen in de eerste drie maanden na de geboorte nodig had (83% versus 87%). Bovenstaande resultaten bieden daarom geen ondersteuning voor het gebruik van IVIg bij neonaten met Rhesus hemolytische ziekte. Met het oog op het ontbreken van positieve effecten, moet het gebruik van IVIg voor deze indicatie dan ook worden ontmoedigd.

In **Hoofdstuk 4** rapporteren we een à terme pasgeborene met Rhesus hemolytische ziekte die werd behandeld met een wisseltransfusie via een katheter in de navelstrengader. Dit kind ontwikkelde hersenabcessen als gevolg van een Bacillus cereus sepsis. Deze ernstige complicatie is niet eerder beschreven. We bespreken mogelijke oorzaken voor deze infectie, het mogelijke verband met de wisseltransfusie en suggesties voor preventie.

In **Hoofdstuk 5** bespreken we de resultaten van een studie over complicaties gerelateerd aan wisseltransfusies. Zoals eerder vermeld, is een wisseltransfusie een risicovolle, invasieve procedure waarbij gebruik gemaakt wordt van centrale lijnen in de bloedbaan (bijvoorbeeld in een navelstrengader). Eerder gerapporteerde bijwerkingen van wisseltransfusies omvatten hoofdzakelijk katheter-gerelateerde complicaties, metabole stoornissen (van elektrolieten), hematologische complicaties en cardio-respiratoire problemen. Om te

onderzoeken wat het ziekte- en sterftepercentage ten gevolge van wisseltransfusies was op onze afdeling, hebben we een grote serie pasgeborenen met Rhesus hemolytische ziekte bestudeerd, die opgenomen waren geweest op onze afdeling. We registreerden het aantal complicaties tijdens opname in de groep van pasgeborenen behandeld met en zonder wisseltransfusie. Van totaal 347 kinderen met HZFP die geanalyseerd werden, werd 39% behandeld met ten minste één wisseltransfusie tijdens opname (ET-groep) en in 61% werd geen wisseltransfusie gegeven (niet-ET-groep). Vergelijking tussen de ET-groep en niet-ET-groep toonde aan dat behandeling met wisseltransfusie onafhankelijk geassocieerd was met: bewezen sepsis (respectievelijk 8% versus 1%), leukopenie (te kort aan witte bloedcellen) (88% versus 23%), ernstige trombocytopenie (te kort aan bloedplaatjes) (trombocytenaantal < 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%). Dit waren allemaal voorbijgaande problemen. Neonatale sterfte kwam niet voor in de groep die behandeld werd met wisseltransfusie. Concluderend kunnen we stellen dat in ervaren handen ernstige blijvende morbiditeit en mortaliteit ten gevolge van wisseltransfusie-procedures tot een minimum kan worden beperkt.

Hoofdstuk 6 bespreekt het probleem van cholestase, een regelmatig waargenomen complicatie van Rhesus hemolytische ziekte. Cholestase is een leverziekte waarbij er een te hoge concentratie geconjugeerd (gebonden) bilirubine in de bloedbaan is. Etiologie van cholestatische leverziekte bij neonaten met HZFP wordt in verband gebracht met ijzerstapeling als gevolg van rode bloedceltransfusies aan de foetus in de baarmoeder (intra-uteriene transfusies, IUT). Gegevens over de incidentie en de ernst van cholestase bij pasgeborenen met HZFP zijn schaars en er is weinig bekend over pathogenese, risicofactoren, neonatale behandeling en uitkomst. We bestudeerden retrospectief een grote groep van 313 kinderen met Rhesus hemolytische ziekte die behandeld waren met of zonder IUT en na de geboorte opgenomen op onze afdeling. We zagen dat cholestase voorkwam bij 13% van de pasgeborenen en dat het onafhankelijk geassocieerd was met behandeling met IUT en met het hebben van Rhesus D-type alloimmunisatie. Hoewel de cholestase meestal mild en van voorbijgaande aard was, waren er enkele pasgeborenen met een ernstige cholestatische leverziekte met langdurig beloop, waarbij intensieve behandeling en in één geval ontijzeringstherapie nodig was. We concluderen daarom dat grotere follow-up studies zijn nodig om het exacte beloop en de etiologie van cholestase vast te stellen bij kinderen met HZFP.

In **Hoofdstuk 7** beschrijven we een andere veel voorkomende complicatie van HZFP, namelijk trombocytopenie bij de geboorte. Beperkte studies hebben aangetoond dat foetussen met Rhesus hemolytische ziekte een verhoogd risico hebben op trombocytopenie. Echter, de incidentie, ernst en risicofactoren van trombocytopenie bij de geboorte

zijn onduidelijk. Daarom hebben we retrospectief 362 neonaten met HZFP onderzocht, die opgenomen waren geweest op onze afdeling. We hebben bloedplaatjes gemeten bij de geboorte en de incidentie van trombocytopenie (< $150x10^9$/L), ernstige trombocytopenie (< $50x10^9$/L) en risicofactoren voor trombocytopenie geëvalueerd. Wij zagen dat trombocytopenie voorkwam in 26% van de pasgeborenen met HZFP bij de geboorte. Ernstige trombocytopenie werd gevonden in 6% van de pasgeborenen. Slechts één pasgeborene met trombocytopenie had klinische symptomen van een hersenbloeding bij de geboorte (intraventriculaire bloeding graad 2). Hoewel deze neonaat trombocytopenie had bij de geboorte, hebben zeer waarschijnlijk ook andere factoren, zoals prematuriteit en hydrops bijgedragen aan het krijgen van een bloedingscomplicatie. We vonden dat drie risicofactoren onafhankelijk van elkaar geassocieerd waren met trombocytopenie bij de geboorte, namelijk behandeling met IUT, dysmaturiteit (te klein voor de zwangerschapsduur) en prematuriteit (lagere zwangerschapsduur bij de geboorte).

In **Hoofdstuk 8** bestudeerden we het effect van een restrictieve richtlijn voor het geven van wisseltransfusies op het aantal top-up transfusies (rode bloedceltransfusies) bij neonaten met HZFP in de eerste drie maanden na de geboorte. In december 2005 veranderden we ons wisseltransfusie-beleid (volgens de aanbevelingen van de American Academy of Pediatrics) van het gebruik van liberale wisseltransfusie-criteria naar meer restrictieve wisseltransfusie-criteria. In deze studie hebben we 183 (bijna) à terme neonaten met HZFP geïncludeerd, allen opgenomen op onze afdeling. We registreerden het aantal wisseltransfusies en het aantal top-up transfusies in de groep van pasgeborenen vóór (groep I, n = 156) en na (groep II, n = 27) de wijziging van de richtlijn. Het percentage van pasgeborenen bij wie een wisseltransfusie werd gegeven, daalde aanzienlijk van 66% in groep I naar 26% in groep II. Het percentage dat een top-up transfusie kreeg, was gestegen van 68% in groep I tot 81% in groep II. We concludeerden dat meer restrictieve wisseltransfusie-criteria bij neonaten met HZFP leiden tot een vermindering van het aantal wisseltransfusies, maar een toename van het aantal top-up transfusies.

Het doel van de studie beschreven in **Hoofdstuk 9** was om neonatale uitkomsten en hematologische complicaties te evalueren in een grote serie van neonaten met Kell hemolytische ziekte vergeleken met neonaten met Rhesus D hemolytische ziekte. Kell alloimmunisatie speelt na Rhesus D de belangrijkste rol in het ontstaan van door antistof veroorzaakte foetale bloedarmoede. Het mechanisme van het ontstaan van foetale bloedarmoede bij Kell hemolytische ziekte is anders dan bij Rhesus D hemolytische ziekte. Bij Kell hemolytische ziekte ligt de nadruk niet zozeer op de afbraak (hemolyse) van de rode bloed cellen maar op de aanmaak van nieuwe rode bloed cellen. De aanmaak is namelijk sterk onderdrukt en kan leiden tot ernstige foetale bloedarmoede. Daardoor is de neonatale behandeling van pasgeborenen met

Kell hemolytische ziekte anders dan bij Rhesus D hemolytische ziekte. Doordat de hemolyse minder ernstig verloopt, hebben neonaten met Kell hemolytische ziekte ook minder last van hyperbilirubinemie. Wel hebben ze, net als bij Rhesus D hemolytische ziekte, last van bloedarmoede en moeten regelmatig met een top-up transfusie behandeld worden. In deze studie hebben we 191 pasgeborenen geïncludeerd en we zagen dat neonaten met Kell-antistoffen minder fototherapie nodig hadden (2,4 versus 4,1 dagen) en minder wisseltransfusies (6% versus 62%) vergeleken met neonaten met Rhesus D hemolytische ziekte. Echter, de behoefte aan top-up transfusies was vergelijkbaar in beide groepen (62% versus 72%).

De lange termijn neurologische uitkomsten bij kinderen met HZFP, die behandeld zijn met IUT, worden gepresenteerd in **Hoofdstuk 10**. De behandeling met IUT is tegenwoordig één van de meest succesvolle procedures in de foetale therapie, met een perinatale overleving van meer dan 95% in ervaren centra. Echter, één van de zorgen omtrent het succesvolle gebruik van foetale therapie is dat een daling van de perinatale sterfte kan leiden tot een toename van kinderen met lange termijn handicaps en/of ontwikkelingsachterstand. Slechts een paar studies met kleine aantallen patiënten hebben in het verleden gekeken naar lange termijn neurologische uitkomsten na IUT. Daarom is de LOTUS studie opgezet. Het doel van deze studie was om de incidentie en risicofactoren van afwijkende psychomotorische ontwikkeling na behandeling met IUT te bepalen. Psychomotorische uitkomst bij kinderen van tenminste 2 jaar oud werd beoordeeld met behulp van gestandaardiseerde tests, waaronder de 'Bayley Scales of Infant Development', de 'Wechsler Preschool and Primary Scale of Intelligence' en de 'Wechsler Intelligence Scale for Children', afhankelijk van de leeftijd van het kind. Primaire uitkomstmaat was de incidentie van afwijkende psychomotorische ontwikkeling, gedefinieerd als tenminste één van de volgende: spasticiteit, ernstige vertraging in de ontwikkeling en bilaterale doofheid en/of blindheid. In totaal werden 291 kinderen onderzocht met een mediane leeftijd van 8,2 jaar, variërend van 2 tot 17 jaar. Spasticiteit kwam in 2,1% van de kinderen voor, ernstige ontwikkelingsachterstand in 3,1% en bilaterale doofheid in 1,0%. De totale incidentie van ernstige afwijkende neurologische ontwikkeling was 4,8%. We vonden ook dat ernstige hydrops onafhankelijk geassocieerd was met afwijkende psychomotorische ontwikkeling. We concludeerden dat preventie van foetale hydrops door tijdige detectie, verwijzing en behandeling, de lange termijn uitkomst nog verder kan verbeteren.

Concluderend kunnen we stellen dat perinatale morbiditeit en mortaliteit van rode bloedcel alloimmunisatie sterk is afgenomen de laatste 50 jaren als gevolg van een significante vooruitgang in pre- en postnatale zorg en behandeling. Echter, een aantal vragen omtrent de behandeling en complicaties van HZFP blijft nog onbeantwoord en dit vormt de basis voor het doen van toekomstig onderzoek.

List of abbreviations

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AAP	American Academy of Pediatrics
BSID	Bayley Scales of Infant Development
CI	Confidence interval
CP	Cerebral palsy
EPO	Erythropoietin
ET(s)	Exchange transfusion(s)
Hb	Hemoglobulin
HDFN	Hemolytic disease of the fetus/newborn
HDN	Hemolytic disease of the neonate/newborn
IgG	Immunoglobulin
IQR	Interquartile range
IUT(s)	Intrauterine transfusion(s)
IVIg	Intravenous immunoglobulin
LIVIN	**L**eiden's **IVI**g trial in Rhesus disease of the **N**eonate
LOTUS	**LO**ng-**T**erm follow-up after intra**U**terine transfusion**S**
LUMC	Leiden University Medical Center
MDI	Mental developmental index
MND	Minor neurological dysfunction
NDI	Neurodevelopmental impairment
OR	Odds ratio
PT	Phototherapy
RCT	Randomized controlled trial
Rh	Rhesus
Rh D	Rhesus D
RHDN	Rhesus hemolytic disease of the neonate/newborn
SD	Standard deviation
WISC	Dutch version of Wechsler Intelligence Scale for Children
WPPSI	Dutch version of Wechsler Preschool and Primary Scale of Intelligence

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