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Hematological outcome in neonatal alloimmune hemolytic disease

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Chapter 2

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

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Introduction

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

Anemia

Early versus late anemia: etiology

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

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

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

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

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

Although late anemia was already described before the use of IUT, treatment with IUT seems to increase and prolong the risk of anemia, probably by reducing erythropoiesis.⁴ A high persistent antibody titer in Rhesus D hemolytic disease can also contribute to prolonged anemia.⁵ In contrast, in Kell HDFN antibody titer does not correspond well with the severity of anemia. Anemia in Kell HDFN seems to result from reduced erythropoiesis caused by destruction of progenitor red blood cells rather than hemolysis of erythrocytes.⁶ Although fetal anemia in Kell HDFN is often more severe necessitating more often IUT treatment compared to Rhesus D HDFN, in the neonatal period no significant differences

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

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

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

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

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

Top-up transfusions

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

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

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

Erythropoietin

Some authors advocate the use of Erythropoietin (EPO) to prevent late anemia in neonates with HDFN.¹⁰⁻¹⁹ EPO is a glycoprotein hormone responsible for fetal and neonatal erythropoiesis and increases on hypoxic stimuli. The normal EPO-level in the non-anemic full-term infants at 7-50 days of age was estimated to be 30 mU/ml.³ In the fetal period EPO is produced in the liver and does not cross the placenta and after birth production shifts to the kidneys. In HDFN EPO-production can be insufficient for the degree of anemia.¹⁸ Since 1992 several reports on the use of recombinant human erythropoietin (rhEPO) in

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

Unfortunately effective rhEPO treatment can only be administered subcutaneously and intravenously.

Long-acting erythropoietin

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

Supplements to support erythropoiesis

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

Folic acid

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

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

Folic acid dosages reported in the literature vary from 0.025 to 5 mg/d and side effects (such as rash, fever) are uncommon.

Even though high-level evidence is lacking, in our centre we routinely supplement 0.05 mg/d orally to infants with HDFN during the first three months of life.

Iron

Iron supplementation is also used to support erythropoiesis in anemic neonates with HDFN. However, neonates with HDFN usually do not lack iron. On the contrary, in Rhesus HDFN ferritin levels (which reflects total body iron stores) in fetal and cord blood are highly elevated.^{27,28} Moreover, in HDFN blood transfusions are frequently necessary and with each top-up transfusion, iron is transfused as well. Multiple intrauterine and/or postnatal blood transfusions can lead to iron overload that exceeds transferrin binding capacity. Non-transferrin bound iron can lead to the formation of hydroxyl radicals which can damage lipids, proteins, sugars and DNA.²⁷ Additionally, iron overload can cause damage to the liver, heart and endocrine organs and also can alter immune response and increase susceptibility to infection.²⁹ Nevertheless, iron is crucial for early brain growth and function and iron deficiency as well as iron overload have been associated with neurodevelopmental impairment.²⁹

The incidence and morbidity of iron overload and long-term neurodevelopmental outcome in HDFN is unclear. Therefore, iron supplementation should be withheld, especially in transfused infants with HDFN, unless ferritin levels are in the low/normal ranges. More research is necessary to define indications for chelation therapy, which occasionally is used to treat iron overload in HDFN.³⁰

Vitamin E

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

Exchange transfusion

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

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

Treatment with intravenous immunoglobulin (IVIg)

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

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

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

Thrombocytopenia

Incidence of thrombocytopenia in HDFN

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

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

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

Etiology of thrombocytopenia in HDFN

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

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

Coagulation

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

Leucopenia

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

Key guidelines

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

Research directions

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

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