Neonatal management and outcome in red cell alloimmunization

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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.

References

1. Smits-Wintjens VE, Walther FJ, Lopriore E: Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. *Semin Fetal Neonatal Med* 2008;13:265-271.
2. Allgood C, Bolisetty S: Severe conjugated hyperbilirubinaemia and neonatal haemolysis. *Int J Clin Pract* 2006;60:1513-1514.
3. Dunn PM: Rh Haemolytic Disease of the Newborn, 1960-1961. Arch Dis Child 1963;38:596-599.
4. Dunn PM: Obstructive Jaundice and Haemolytic Disease of the Newborn. *Arch Dis Child* 1963;38:54-61.
5. Perez EM, Cooper TR, Moise AA, Ferry GD, Weisman LE: Treatment of obstructive jaundice in erythroblastosis fetalis with ursodeoxycholic acid (UDCA): a case report. *J Perinatol* 1998;18:317-319.
6. Lasker MR, Eddleman K, Toor AH: Neonatal hepatitis and excessive hepatic iron deposition following intrauterine blood transfusion. *Am J Perinatol* 1995;12:14-17.
7. Yilmaz S, Duman N, Ozer E, Kavas N, Oren H, Demircioglu F, Kumral A, Ozkan H, Irken G, Ozer E: A case of rhesus hemolytic disease with hemophagocytosis and severe iron overload due to multiple transfusions. *J Pediatr Hematol Oncol* 2006;28:290-292.
8. Aygun C, Tekinalp G, Gurgey A: Increased fetal iron load in rhesus hemolytic disease. *Pediatr Hematol Oncol* 2004;21:329-333.
9. Berger HM, Lindeman JH, van Zoeren-Grobben D, Houdkamp E, Schrijver J, Kanhai HH: Iron overload, free radical damage, and rhesus haemolytic disease. *Lancet* 1990;335:933-936.
10. Nasrat HA, Nicolini U, Nicolaidis P, Letsky EA, Gau G, Rodeck CH: The effect of intrauterine intravascular blood transfusion on iron metabolism in fetuses with Rh alloimmunization. *Obstet Gynecol 1991*;77:558-562.
11. De Boer IP, Zeestraten EC, Lopriore E, Van Kamp IL, Kanhai HH, Walther FJ: Pediatric outcome in Rhesus hemolytic disease treated with and without intrauterine transfusion. *Am J Obstet Gynecol* 2008;198:54.e1-54.e4.

12. Rath ME, Smits-Wintjens VE, Lindenburg I, Brand A, Oepkes D, Walther FJ, Lopriore E: Top-up transfusions in neonates with Rh hemolytic disease in relation to exchange transfusions. *Vox Sang* 2010;99: 65-70.
13. Rath ME, Smits-Wintjens VE, Lindenburg IT, Brand A, Van Kamp IL, Oepkes D, Walther FJ, Lopriore E: Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. *Vox Sang* 2011;100:312-316.
14. Smits-Wintjens VE, Walther FJ, Rath ME, Lindenburg IT, te Pas AB, Kramer CM, Oepkes D, Brand A, Lopriore E: Intravenous immunoglobulin in neonates with rhesus hemolytic disease: a randomized controlled trial. *Pediatrics* 2011;127:680-686.
15. Moyer V, Freese DK, Whitington PF, Olson AD, Brewer F, Colletti RB, Heyman MB: Guideline for the evaluation of cholestatic jaundice in infants: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2004;39:115-128.
16. Ayling RM, Carragher F: Neonatal biochemical reference ranges (appendix 6) in: Rennie JM, editor. *Roberton's Textbook of Neonatology*, ed 4. Philadelphia, Elsevier, 2005:1299-1308.
17. Roberts IAG: Haematological values in the newborn (appendix 1) in: Rennie JM, editor. *Roberton's Textbook of Neonatology*, ed 4. Philadelphia, Elsevier, 2005:1287.
18. Bowman JM: Another cause of neonatal cholestasis. *J Pediatr* 1986;108:489.
19. Sivan Y, Merlob P, Nutman J, Reisner SH: Direct hyperbilirubinemia complicating ABO hemolytic disease of the newborn. *Clin Pediatr (Phila)* 1983;22:537-538.