Neonatal management and outcome in red cell alloimmunization

Smits-Wintjens, V.E.H.J.

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Thrombocytopenia at birth in neonates with red cell alloimmune hemolytic disease

Mirjam EA Rath and Vivianne EHJ Smits-Wintjens
Dick Oepkes
Erik W van Zwet
Inge L van Kamp
Anneke Brand
Frans J Walther
Enrico Lopriore

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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.

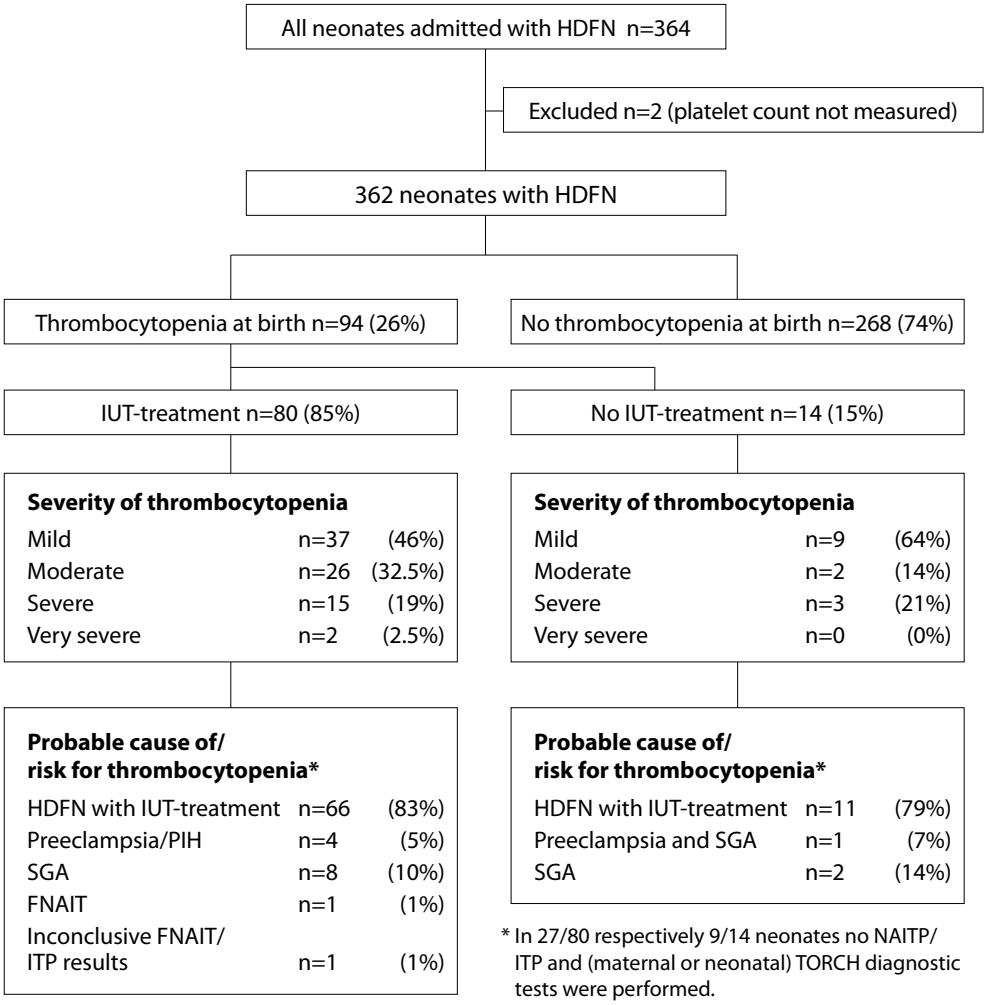
Four percent (14/362) of neonates received a platelet transfusion at birth due to thrombocytopenia.

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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