Optimising neonatal management of haemolytic disease of the foetus and newborn

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Chapter five

Predicting anaemia and transfusion dependency in severe alloimmune haemolytic disease of the foetus and newborn in the first three months after birth

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Abstract

Infants with haemolytic disease of the foetus and newborn (HDFN) often require erythrocyte transfusions the first three months of life. We aimed to evaluate the incidence, timing and potential predictors of transfusion-dependent anaemia. An observational cohort of 298 term and near-term infants with severe HDFN treated with or without intrauterine transfusion (IUT) was evaluated.

Transfusions were administered to 88% (169/193) of infants with IUT and 60% (63/105) without IUT. The following potential predictors were associated with less anaemia: K compared to D immunisation (odds ratio [OR] 0.13, 95%-confidence interval [95% CI]: 0.03-0.55), higher reticulocyte count at birth (per 10 parts per thousand [‰] higher, OR 0.99, 95% CI 0.97-1.00) and exchange transfusion (OR 0.11, 95% CI 0.03-0.50). Without IUT, these variables were: lower reticulocyte count at birth (per 10‰ lower, OR 1.02, 95% CI 1.00-1.03), lower maximum bilirubin after birth (per 10 µmol/L lower, OR 1.01, 95% CI 1.01-1.02) and exchange transfusion (OR 0.07, 95% CI 0.01-0.20).

In conclusion, potential predictors for anaemia in infants with severe HDFN varied between infants treated with and without IUT and are useful to select subgroups of infants at increased risk of anaemia.

Introduction

Haemolytic disease of the foetus and newborn (HDFN) is caused by the destruction of foetal and neonatal erythrocytes and possible impairment of erythropoiesis by maternal erythrocyte alloantibodies. HDFN may result in foetal and neonatal anaemia, which may persist up to three months after birth.[1,2] Prediction of this persisting anaemia after birth and of the duration of the possible transfusion dependency are central issues in the neonatal management of haemolytic disease. Anaemia in infants with HDFN is due to ongoing haemolysis, but also due to depressed erythropoiesis (hyporegenerative anaemia) and ongoing haemolysis.[3-5] While the usually early haemolytic anaemia in HDFN is one of the major focusses of neonatal care of these infants at admission, it is often overlooked how long anaemia and transfusion dependency can persist.

The vast majority of infants with severe HDFN suffer from pronounced anaemia and require erythrocyte transfusions during the first three months after birth.[6] Several factors are associated with an increased transfusion need in HDFN, including intrauterine transfusions (IUTs), treatment with exchange transfusion, severity of HDFN, and type of blood group alloimmunisation.[6-8] IUTs are thought to suppress foetal erythropoiesis and increase the need for transfusions after birth[8], whereas exchange transfusions appear to decrease the need for transfusions.[7] However, which infants are at the highest risk of developing anaemia, the time when anaemia typically presents and how long it can last before full physiologic recovery, are unclear. These questions need to be answered to design optimal follow-up procedures for these infants, finding the balance in close (laboratory) monitoring and excessive blood sampling in an anaemic population.

HDFN is rare and the care for these infants is usually scattered and shows great variability between and within countries and regions. In the Netherlands, treatment of severe HDFN is centralised in our national referral centre. We can therefore report on a considerably sized, nearly complete and to a great extent, homogenously treated population. In this study, we aimed to evaluate the incidence and potential predictors of anaemia in the first three months of life in a population of infants with severe HDFN in order to evaluate the disease trajectory in relation to transfusion management. This knowledge can be used to prevent unnecessary blood sampling and extended diagnostics in this group of infants with increased risk for HDFN-associated anaemia.

Methods

Study population

All term and near-term infants (born ≥35 weeks of gestation) with HDFN admitted to the Leiden University Medical Centre (LUMC) between January 2006 and January 2018 were eligible for the study. The LUMC is the national referral centre for severe HDFN and intrauterine treatment in the Netherlands. The guideline used in the Netherlands as part of the routine erythrocyte antibody screening programme, indicates referral to the LUMC if laboratory parameters are above determined cut-offs. This concerns antibody titres tested in maternal serum ≥2 in K immunisation and ≥16 in D or other types of alloimmunisation, or, in case of an antibody-dependent cell-mediated cytotoxicity (ADCC) assay ≥50% in case of D immunisation and ≥30% in case of other blood group antigens.[9] Subsequently, these high-risk pregnancies are monitored by serial Doppler measurements to assess the velocity of the blood flow in the middle cerebral artery (MCA). If MCA Doppler exceeds 1.5 multiples of the median velocity or if signs of hydrops are present, treatment with IUT is indicated. One or more IUTs can be administered until 34-35 weeks' gestation.[10] In this study, an antenatal diagnosis of HDFN was defined as antibody titres or ADCC test results above the aforementioned cut-offs. In case of missing values, treatment with IUT was also considered as a valid confirmation of clinically relevant HDFN.

We excluded infants born <35 weeks of gestation and infants with incomplete follow-up data regarding anaemia and transfusions after birth, which was the primary outcome measure. We also excluded infants who participated in an ongoing randomised trial on the use of erythropoietin (EPO) to prevent anaemia in HDFN and were randomised to the treatment arm. This study started in October 2017 at the LUMC (EPO-4-Rh study, NCT03104426).

The results of the current study were separately reported for infants treated with and without IUT, since an IUT may influence anaemia occurring after birth. IUT treatment is, by its indication, a sign of very severe illness, but may also alter the (patho)physiological course in these infants, which makes comparison of infants with and without IUT treatment inappropriate. Similarly for D and K alloimmunisation, a different role in the pathogenesis has been described for K alloantibodies, which are correlated to the inhibition of foetal erythropoiesis.[7] Therefore, and taking into consideration the small sample sizes of other types of alloimmunisation, extra analyses were performed excluding all types of alloimmunisation other than D.

Data collection

Data was extracted from the hospital's patient database, including medical files and laboratory outcomes. Follow-up data on transfusions after discharge from the LUMC were collected from referral hospitals after written consent was obtained from the parents or caregivers. All collected data were coded for analysis. The following maternal and neonatal data were recorded: type of alloimmunisation (D, C, c, E or K), treatment with IUT and number of IUTs, gestational age at first IUT, foetal haemoglobin level prior to first IUT, mode of delivery, gestational age at birth, birth weight, neonatal sex, neonatal haemoglobin level, bilirubin level, reticulocyte count at birth, treatment with erythrocyte transfusions and number of transfusions, neonatal age at first transfusion in days, neonatal haemoglobin level prior to first transfusion, and treatment with exchange transfusion. The study protocol and analysis plan were approved by the ethical committee of the LUMC.

Definitions and transfusion policy

As there are no uniform haemoglobin thresholds to define anaemia in newborns, anaemia was defined as a need for one or more erythrocyte transfusions in this study. The current transfusion guideline of the department, implemented in February 2014, recommends a transfusion in term infants with HDFN when haemoglobin levels fall below 10.5 g/dL (6.5 mmol/L) for day 0-6, below 8.9 g/dL (5.5 mmol/L) for day 7-13, and below 7.2 g/dL (4.5 mmol/L) from day 14 onwards. The former guideline recommended transfusion when haemoglobin levels fell below 9.6 g/dL (6.0 mmol/L) for day 7-13, and below 8.0 g/dL (5.0 mmol/L) from day 14 onwards. A transfusion of 15 ml/kg irradiated packed erythrocytes less than 5 days old was advised throughout the study period, with a haematocrit of 0.50-0.65 L/L.

Primary and secondary outcome

Primary outcome was the incidence of infants with HDFN treated for anaemia after birth i.e. those who received one or more erythrocyte transfusions. Secondary outcomes were the mean number of transfusions per infant and the mean number of days after birth before the first transfusion. Furthermore, potential predictors of anaemia were identified in subgroups of HDFN treated with and without IUT(s).

Statistical analysis

Anaemia and transfusion outcomes for infants treated with and without IUT were reported. The following variables known from literature as potential predictors of anaemia were compared

between infants with and without anaemia: sex, gestational age at birth, type of blood group alloimmunisation, treatment with an exchange transfusion, maximum bilirubin after birth, and reticulocyte level at birth. All these variables were also included in a multivariable logistic model. Separate models were run for infants with and without IUT treatment. The type of blood group alloimmunisation is divided in D immunisation, K immunisation and "other".

Results are presented as odds ratios (OR) with 95%-confidence intervals (95% CIs). An area under the curve (AUC) measure of the regression models is reported and a Kaplan-Meier curve depicting the transfusion free interval was presented. Statistical analyses were performed using IBM SPSS Statistics (version 23.0; SPSS Inc, Chicago, IL).

Results

During the studied period, 347 infants with an antenatal diagnosis of HDFN were born and admitted to the neonatal intensive care unit (NICU) of the LUMC. A total of 49 infants were excluded, of which 27 infants were excluded for a gestational age <35 weeks, and 21 infants were excluded based on missing data regarding anaemia and transfusion dependency after discharge from the LUMC. One exclusion was due to participation in the EPO-4-Rh trial and randomisation for EPO treatment. A total of 298 infants fulfilled the eligibility criteria and were included in the study (Figure 1) and follow-up was complete for 93% (277/298) of the infants. Of the 21 infants excluded based on missing data, 7 were treated with IUT and 14 were not treated with IUT, the types of alloimmunisation in this group were as follow: 13 infants were diagnosed with D immunisation, 2 with K, 4 with c, 1 with C and 1 with E.

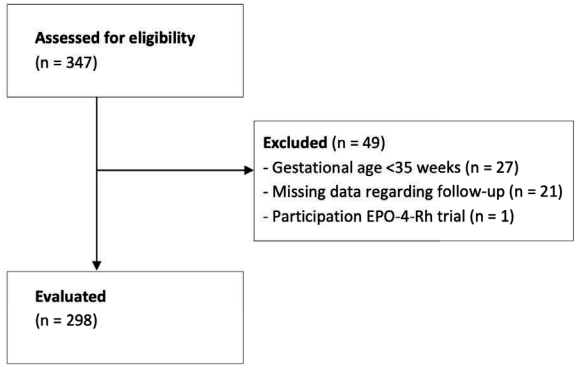

Figure 1. Flowchart of study participants

Baseline characteristics

Baseline characteristics of the cohort are presented in Table 1. The majority of the cohort was treated with IUT (193/298, 65%). Among infants who had been treated with IUT, the mean haemoglobin level at birth was 12.8 g/dL (standard deviation [SD] 2.8), the median reticulocyte count was 18 parts per thousand (‰; interquartile range [IQR] 3-56) and the median number of IUTs was 3 (IQR 2-3). Among infants not treated with IUT the mean haemoglobin level at birth was 14.2 g/dL (SD 3.4) and the median reticulocyte count was 84‰ (IQR 59-134).

Table 1. Baseline characteristics of infants with HDFN

	IUT (n = 193)	**No-IUT (n = 105)**
Male - n (%)	118 (61)	65 (62)
Caesarean delivery - n (%)	43 (22)	21 (20)
Gestational age at birth (weeks) - median (IQR)	36.0 (36.0-37.0)	37.0 (36.0-37.0)
Birth weight (grams) - mean ± SD	2902 ± 369	3000 ± 484
Haemoglobin level at birth (g/dL) - mean ± SD	12.8 ± 2.8[a]	14.2 ± 3.4
Reticulocyte count at birth (‰) - median (IQR)	18 (3-56)[b]	84 (59-134)
Number of IUTs - median (IQR)	3.0 (2.0-4.0)	-
Gestational age at first IUT (weeks) - median (IQR)	28.6 (24.2-31.8)	-
Haemoglobin level at first IUT (g/dL) - mean ± SD	7.1 ± 2.5[c]	-
D alloimmunisation - n (%)	148 (77)	76 (72)
C alloimmunisation - n (%)	0 (0)	2 (2)
c alloimmunisation - n (%)	8 (4)	13 (12)
E alloimmunisation - n (%)	1 (1)	6 (6)
K alloimmunisation - n (%)	35 (18)	4 (4)
Jka alloimmunisation - n (%)	1 (1)	2 (2)
Cw alloimmunisation - n (%)	0 (0)	2 (2)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *SD*, standard deviation.
[a] Assessed in 296/298 (99%) infants**,** 2 cases of missing value; [b] assessed in 280/298 (94%) infants, 18 cases of missing value; [c] assessed in 187/193 (97%) infants, 6 cases of missing value.

Transfusion dependency

Table 2 shows the transfusion dependency in relation to treatment with IUT(s). The incidence of anaemia (i.e. need for transfusions) after birth was 88% among infants treated with IUT and

60% among infants without IUT. The median number of transfusions was 2 (IQR 2-3) among infants with IUT and 2 (IQR 1-3) among infants without IUT. The first transfusion was given after a median of 16 days (IQR 5-27) after birth for infants with IUT, and 9 days (IQR 5-25) after birth for infants without IUT.

In case of D immunisation, IUT was performed in 66% (148/224) and 83% (187/224) received at least one transfusion after birth. In case of K immunisation, 90% (35/39) was treated with IUT and 72% (28/39) was anaemic after birth and received at least one transfusion. In the combined group of C, c and E immunisation, an IUT was performed in 29% (9/30) and 52% (16/30) of infants were treated with a transfusion after birth.

Table 2. Transfusions dependency in infants with HDFN with and without treatment with intrauterine transfusion

	IUT (n = 193)	**No-IUT (n = 105)**
Infants treated with transfusions - n (%)	169/193 (88)	63/105 (60)
Number of transfusions per infant - median (IQR)[a]	2 (2-3)	2 (1-3)
Infants received:		
1 transfusion - n (%)	40/169 (24)	27/63 (43)
2 transfusions - n (%)	62/169 (37)	16/63 (25)
3 transfusions - n (%)	39/169 (23)	12/63 (19)
4 transfusions - n (%)	19/169 (11)	5/63 (8)
5 transfusions - n (%)	5/169 (3)	3/63 (5)
6 transfusions - n (%)	4/169 (2)	0/63 (0)
Days after birth until first transfusion - median (IQR)[a,b]	16 (5-27)	9 (5-25)
Haemoglobin level at first transfusion (g/dL) - median (IQR)[a,c]	7.9 (6.8-8.9)	7.9 (7.2-8.9)
Infants treated with exchange transfusions - n (%)	31/193 (16)	16/105 (15)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number.
[a] In infants treated with transfusion(s), n=232; [b] assessed in 229/232 (99%) infants, 3 cases of missing value; [c] assessed in 223/232 (96%) infants, 9 cases of missing value.

Predictors for anaemia among infants treated with an IUT

In univariable analysis among infants treated with IUT, three variables were associated with less anaemia: K immunisation, compared with D immunisation (16 vs 33%, OR 0.35, 95% CI 0.14 to 0.92), a higher reticulocyte count at birth (10 vs 50‰, OR 0.99, 95% CI 0.98 to 1.00)

and treatment with exchange transfusion (13 vs 38%, OR 0.25, 95% CI 0.10 to 0.64). There were no statistically significant differences between anaemic and non-anaemic infants for sex, D or other types of immunisation, gestational age at birth and maximum bilirubin after birth (Table 3).

In multivariable analysis, the same three variables were associated with less anaemia: K immunisation, compared to D immunisation (OR 0.15, 95% CI 0.04 to 0.59), a higher reticulocyte count at birth (per 10‰ higher, OR 0.99, 95% CI 0.97 to 1.00) and treatment with exchange transfusion (OR 0.11, 95% CI 0.03 to 0.58). The AUC of this model is 0.83, 95% CI 0.76 to 0.91.

Predictors for anaemia among infants not treated with IUT

Among infants who had not been treated with IUT, three variables were associated with less anaemia: occurrence of the combined group of non-D, non-K types of immunisation (15 vs 38%, OR 0.24, 95% CI 0.09 to 0.63), a lower reticulocyte count at birth (100 vs 60‰, OR 1.01, 95% CI 1.00 to 1.02), and a lower maximum bilirubin after birth (290 vs 260 µmol/L, OR 1.01, 95% CI 1.00 to 1.02). The other variables, sex, D or K alloimmunisation, and treatment with exchange transfusion, were not statistically associated with anaemia after birth (Table 4).

In multivariable analysis, three variables were associated with less anaemia: a lower reticulocyte count at birth (per 10‰ lower, OR 1.02, 95% CI 1.00 to 1.03), a lower maximum bilirubin count after birth (per 10 µmol/L lower, OR 1.01, 95% CI 1.01 to 1.02), and treatment with exchange transfusion (OR 0.07, 95% CI 0.01 to 0.20). The AUC of this model is 0.83, 95% CI 0.75 to 0.91.

Table 3. Predictors for anaemia in infants with HDFN in infants treated with intrauterine transfusion

	Transfusion (n = 169)	No transfusion (n = 24)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	105/169 (62)	13/24 (54)	1.39 (0.59-3.28)	1.75 (0.63-4.82)
Type of blood group alloimmunisation				
D - n (%)	134/169 (79)	14/24 (58)		
K - n (%)	27/169 (16)	8/24 (33)	0.35 (0.14-0.92)[b]	0.13 (0.03-0.55)
Other - n (%)	8/169 (5)	2/24 (8)	0.42 (0.08-2.16)[b]	0.48 (0.08-2.91)
Gestational age at birth (per week)[a]	36.0 (36.0-37.0)	37.0 (36.0-37.0)	0.61 (0.56-1.06)	0.73 (0.38-1.39)
Reticulocyte count at birth (per 10‰)[a]	10 (0-50)	50 (10-80)	0.99 (0.98-1.00)	0.99 (0.97-1.00)
Maximum bilirubin after birth (per 10 µmol/L)[a]	220 (165-280)	235 (150-308)	1.00 (0.99-1.00)	1.00 (0.99-1.01)
Exchange transfusion - n (%)	22/169 (13)	9/24 (38)	0.25 (0.10-0.64)	0.11 (0.03-0.50)
Number of IUTs (per IUT)[a]	3 (2-4)	2 (1-3)	1.33 (0.92-1.91)	1.29 (0.78-2.13)

CI, confidence interval; *HDFN*, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (IQR) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses; [b] as compared to the risk of anaemia in D immunisation.

Table 4. Predictors for transfusions in infants with HDFN in infants not treated with intrauterine transfusion

	Transfusion (n = 63)	No transfusion (n = 42)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	40/63 (64)	25/42 (60)	1.18 (0.53-2.64)	1.56 (0.56-4.39)
Type of blood group alloimmunisation				
D - n (%)	53/63 (84)	23/42 (55)		
K - n (%)	1/63 (2)	3/42 (7)	0.15 (0.01-1.47)[b]	0.41 (0.01-26.19)
Other - n (%)	9/63 (15)	16/42 (38)	0.24 (0.09-0.63)[b]	0.31 (0.09-1.04)
Gestational age at birth (per week)[a]	37.0 (36.0-37.0)	37.0 (36.0-38.0)	0.66 (0.40-1.08)	1.05 (0.57-1.92)
Reticulocyte count at birth (per 10‰)[a]	100 (60-140)	60 (40-93)	1.01 (1.00-1.02)	1.02 (1.00-1.03)
Maximum bilirubin after birth (per 10 µmol/L)[a]	290 (260-310)	260 (178-295)	1.01 (1.00-1.02)	1.01 (1.01-1.02)
Exchange transfusion - n (%)	7/63 (11)	9/42 (21)	0.46 (0.16-1.35)	0.07 (0.01-0.20)

CI, confidence interval; *HDFN*, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (IQR) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses; [b] as compared to the risk of anaemia in D immunisation.

Transfusion-free survival

Among infants treated with IUT, 12% did not develop anaemia with a need for transfusion (Table 2). The Kaplan-Meier curve presents the transfusion-free interval (Figure 2), which showed a steep decline of the risk for a first transfusion towards 45 days after birth. In this group 97% (188/193) had a first transfusion before 45 days after birth, the median age a first transfusion was given was 16 days, as mentioned above. The highest age was 55 days. The highest age at which a transfusion was given in infants treated with IUT that received multiple transfusions, was exactly 100 days after birth, with a median of 49 days (IQR 44-54). The median transfusion interval was therefore from 16 to 49 days after birth.

Among infants not treated with IUT, 40% did not develop anaemia with a need for transfusion (Table 2). Again the Kaplan-Meier curve showed a decline of the risk for a first transfusion towards 45 days after birth. In this group, 99% (104/105) had a first transfusion before 45 days after birth, the median age at which a first transfusion was given was 9 days. The highest age was 61 days. The highest age at which a transfusion was given in infants not treated with IUT that received multiple transfusions, was 83 days after birth, with a median of 30 days (IQR 26-34). The median transfusion interval was therefore from 9 to 30 days after birth.

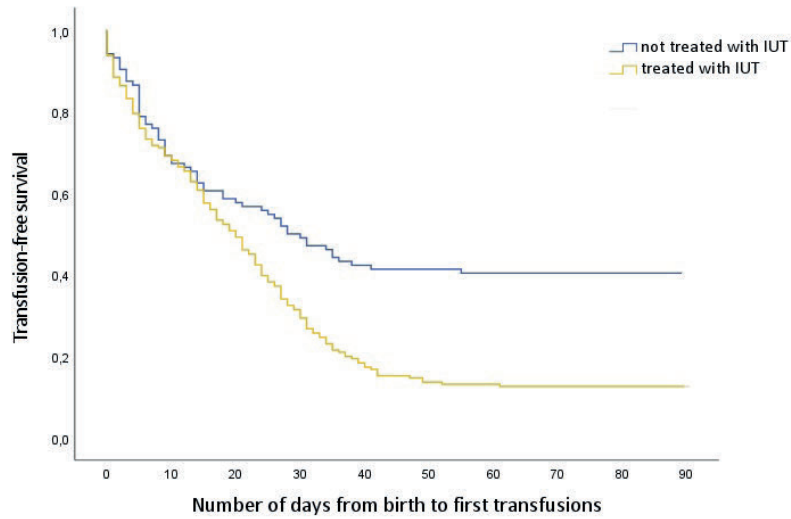

Figure 2. Kaplan-Meier curve, transfusion-free interval
IUT; intrauterine transfusion.

D-immunisation

As most infants had HDFN due to anti-D immunisation (75% of the study population), additional analyses were done after exclusion of other types of immunisation to verify the robustness of our findings in a more homogeneous population (Tables 5-8). In univariable analysis, a higher reticulocyte count at birth (10 vs 80 ‰, OR 0.97, 95% CI 0.96 to 0.99), maximum bilirubin after birth (245 vs 285 µmol/L, OR 0.99, 95% CI 0.99 to 1.00), and treatment with exchange transfusion (16 vs 57%, OR 0.14, 95% CI 0.04 to 0.44) were statistically significantly associated with less anaemia in the subgroup of D immunised pregnancies treated with IUT(s), Table 7. In multivariable analysis, the reticulocyte count at birth remained statistically associated with anaemia and transfusion dependency (OR 0.98, 95% CI 0.96 to 1.00). Treatment with exchange transfusion was not statistically significant in this subpopulation on multivariable analysis.

Among infants not treated with IUT, univariable analysis showed no variables that were statistically significant associated with anaemia in the D-immunised subgroup. Multivariable analysis showed an association between a lower reticulocyte count at birth (per 10‰ lower, OR 1.01, 95% CI 1.00 to 1.02), lower maximum bilirubin after birth (per 10 µmol/L lower, OR 1.01, 95% CI 1.00 to 1.02), and exchange transfusion (OR 0.12, 95% CI 0.02 to 0.58), Table 8.

Table 5. Baseline characteristics of infants with HDFN due to D immunisation

	IUT (n = 148)	**No-IUT (n = 76)**
Male - n (%)	91 (62)	48 (63)
Caesarean delivery - n (%)	34 (23)	18 (24)
Gestational age at birth (weeks) - median (IQR)	36.0 (36.0-37.0)	37.0 (36.0-37.0)
Birth weight (grams) - mean ± SD	2859 ± 367	3075 ± 479
Haemoglobin level at birth (g/dL) - mean ± SD	12.7 ± 2.8[a]	13.7 ± 3.4
Reticulocyte count at birth (‰) - median (IQR)	22 (3-58)[b]	90 (65-134)
Number of IUTs - median (IQR)	2.0 (2.0-4.0)	-
Gestational age at first IUT (weeks) - median (IQR)	29.1 (24.6-32.1)	-
Haemoglobin level at first IUT (g/dL) - mean ± SD	7.2 ± 2.5[c]	-

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number; *SD*, standard deviation.
[a] Assessed in 222/224 (99%) infants, 2 cases of missing value; [b] assessed in 214/224 (96%) infants, 10 cases of missing value; [c] assessed in 143/148 (97%) infants, 5 cases of missing value.

Table 6. Transfusion dependency in infants with HDFN due to D immunisation with and without treatment with intrauterine transfusion

	IUT (n = 148)	**No-IUT (n = 76)**
Infants treated with transfusions - n (%)	134/148 (91)	53/76 (70)
Number of transfusions per infant - median (IQR)[a]	2 (2-3)	2 (1-3)
Infants received:		
1 transfusion - n (%)	23/134 (17)	23/53 (43)
2 transfusions - n (%)	50/134 (37)	13/53 (25)
3 transfusions - n (%)	35/134 (24)	10/53 (19)
4 transfusions - n (%)	17/134 (11)	5/53 (9)
5 transfusions - n (%)	5/134 (3)	2/53 (4)
6 transfusions - n (%)	4/134 (3)	0/53 (0)
Days after birth until first transfusion - median (IQR)[a,b]	16 (4-27)	9 (4-21)
Haemoglobin level at first transfusion (g/dL) - median (IQR)[a,c]	7.9 (6.9-9.0)	7.8 (7.1-8.8)
Infants treated with exchange transfusions - n (%)	29/148 (20)	14/76 (18)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *IUT*, intrauterine transfusion; *n*, number.
[a] In infants treated with transfusion(s) after birth, n=187; [b] assessed in 184/187 (98%) infants, 3 cases of missing value; [c] assessed in 179/187 (96%) infants, 8 cases of missing value.

Table 7. Predictors for anaemia in infants with HDFN due to D immunisation in infants treated with intrauterine transfusion

	Transfusion (n = 134)	No transfusion (n = 14)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	8/134 (62)	8/14 (57)	1.22 (0.40-3.72)	1.63 (0.39-6.77)
Gestational age at birth (per week)[a]	36.0 (36.0-37.0)	37.0 (36.0-37.0)	0.46 (0.21-0.99)	0.45 (0.19-1.01)
Reticulocyte count at birth (per 10‰)[a,]	10 (0-50)	80 (48-100)	0.97 (0.96-0.99)	0.98 (0.96-1.00)
Maximum bilirubin after birth (per 10 µmol/L)[a]	245 (180-290)	285 (230-343)	0.99 (0.99-1.00)	1.00 (0.99-1.01)
Exchange transfusion - n (%)	21/134 (16)	8/14 (57)	0.14 (0.04-0.44)	0.26 (0.05-1.43)
Number of IUTs (per IUT)[a]	2 (2-4)	2 (1-2)	1.74 (1.00-3.00)	1.48 (0.72-3.05)

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (*IQR*) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses.

AUC = 0.90 (95% CI 0.81 to 0.98)

Table 8. Predictors for anaemia in infants with HDFN due to D immunisation in infants not treated with intrauterine transfusion

	Transfusion (n = 53)	No transfusion (n = 23)	Univariable OR (95% CI)	Multivariable OR (95% CI)
Male - n (%)	35/53 (66)	13/23 (57)	1.50 (0.55-4.07)	2.01 (0.66-6.14)
Gestational age at birth (per week)[a]	37.0 (36.0-37.0)	37.0 (36.0-38.0)	0.89 (0.50-1.58)	1.06 (0.57-1.95)
Reticulocyte count at birth (per 10‰)[a]	90 (65-130)	80 (60-110)	1.01 (1.00-1.02)	1.01 (1.00-1.02)
Maximum bilirubin after birth (per 10 µmol/L)[a]	280 (260-310)	270 (230-340)	1.00 (1.00-1.01)	1.01 (1.00-1.02)
Exchange transfusion - n (%)	7/53 (13)	7/23 (30)	0.35 (0.11-1.15)	0.12 (0.02-0.58)

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *n*, number; *OR*, odds ratio.
[a] Raw data presented as median (*IQR*) and ORs calculated on raw data, *p*-value presented of data after logarithmic transformation (gestational age and bilirubin count) or after square root transformation (reticulocyte count, due to occurrence of the value zero) to achieve normal distribution in univariable and multivariable analyses

AUC = 0.77 (95% CI 0.66 to 0.88)

Discussion

In this study we quantified the need for erythrocyte transfusions in infants suffering from severe HDFN in relation to treatment with and without IUT(s) and identified potential predictors for anaemia and ongoing transfusion dependency. The overall occurrence of anaemia after birth was 78%. We differentiated between infants treated with and without IUT and analysed the time-dependent pattern of anaemia and transfusion in these infants. Among infants treated with IUT, the incidence of anaemia was 88% and the first transfusion was administered 16 days after birth. Among infants not treated with IUT, the incidence of anaemia was 60% and the first transfusion was administered 9 days after birth. In both groups, transfusion management only incidentally started at a later time point than 45 days.

As part of the pathophysiology of HDFN, it has been described that the ongoing destruction of foetal erythrocytes is compensated by extramedullary haematopoiesis and an erythroblastosis in the infant's blood. Indeed, in the infants that did not receive IUT(s), a *high* reticulocyte count at birth was strongly associated with anaemia. Also, a higher maximum bilirubin count after birth was significantly associated with anaemia. We hypothesise that in infants not treated with IUT(s), a high reticulocyte count at birth reflects an active compensatory mechanism for ongoing haemolysis (supported by higher maximum bilirubin counts after birth) and thus indicates more severe haemolytic illness in this group of non-treated infants than those infants with a low reticulocyte count at birth, resulting in a high transfusion dependency. Among infants treated with IUT, we found that the reticulocyte counts at birth showed an inverse association. After IUT, a *low* reticulocyte count at birth was strongly associated with anaemia. Treatment with IUT(s), by definition, indicates a more severe foetal haemolytic course of HDFN, possibly explaining the more severe neonatal disease trajectory with anaemia and a long period of transfusion dependency. IUTs have been described to suppress erythropoiesis[8,11] and we indeed found a *low* reticulocyte count at birth after IUT treatment. Previous studies also showed a correlation between a lower reticulocyte count at birth and anaemia.[8,12,13] However, low reticulocyte counts are seen in both K and D alloimmunisation, while K alloimmunisation shows a less severe course of anaemia after birth despite a higher IUT need.[7] The possible suppressive effect of IUTs can therefore not fully explain the observed anaemia and long transfusion dependency. A potential explanation could be that in K alloimmunisation even low titres are associated with severe impairment of the foetal erythropoiesis[14] and these infants may therefore show a more rapid decline in antibodies after birth and shorter period of transfusion dependency, despite treatment with IUT(s). D alloimmunisation is associated with high antibody titres, which could explain the prolonged suppressive effects of IUT

treatment, especially in absence of treatment with exchange transfusion that could reduce the high antibody load. Unfortunately, the anti-RBC antibody titres were not routinely sampled in the infants after birth, preventing further speculations. In future studies, prospective measurements of anti-RBC antibody titres could yield additional crucial information.

In multivariable analysis in both the IUT-treated infants and in the infants without IUT, a consistent statistically significant association was found between treatment with exchange transfusion and anaemia. The rate of exchange transfusion among infants treated with and without IUT (19% and 16%) was similar to findings of other recent studies conducted after implementation of the guideline by the American Academy of Pediatrics (AAP) in 2004.[15-18] Exchange transfusions are indicated to remove excessive bilirubin from the neonatal circulation if intensive phototherapy is insufficient. Exchange transfusions have additional effects as it are also erythrocyte transfusions in itself and remove maternal antibodies and IgG coated erythrocytes from the neonatal circulation. Removal of antibodies may theoretically reduce the ongoing process of haemolysis and therefore reduce the risk of anaemia.

The Kaplan-Meier analysis to determine the transfusion-free interval is of high clinical relevance, as it illustrates how the risk of a first transfusion after birth decreased over time and stabilised after approximately 45 days for the vast majority of the infants treated with IUT (97%) and those not receiving IUT (99%). The current expert opinion in the Netherlands is to closely follow-up on haemoglobin and reticulocyte counts on a weekly basis up to three months of life. For this study population, this means that weekly blood sampling test for haemoglobin measurements were performed in 22% of infants that never developed anaemia. Our data suggest that a follow-up period of approximately 45 days is sufficient if, by that time, no transfusions have been necessary. We recommend a shortened follow-up period for infants in good clinical condition, with proper instruction to caregivers.

Overall the study has several strengths and limitations. One of the major strengths is the Dutch setting which enabled us to establish a large, homogenous and near complete collection of data and follow-up records of an increasingly rare disease. However, the results must be carefully interpreted in the context of our population, as it is a selection of (very) severe HDFN cases as result of the referral guidelines in the Netherlands. Infants not treated with IUT, particularly the less severe cases that did not require foetal therapy, were probably more likely to have been admitted elsewhere. It may also be difficult to translate these results to other populations, as there is a great international variability in haemoglobin transfusion thresholds.[19] Other limitations of this study are the small numbers of alloimmunisation other

than D. Additionally, this study did not take other causes of anaemia into consideration, such as infection or blood loss due to obstetric causes or bleeding in the neonatal period,[20] although these were rare in this population. The effect of the most frequent cause of anaemia in infants, prematurity, was eliminated in the study by assessing a (near) term cohort.

In conclusion, severe anaemia is a common complication of HDFN and clinicians need to be aware of this risk and actively monitor the process of haemolysis and recuperation of erythropoiesis in HDFN. IUT treatment, by its definition and indications, reflects a severe haemolytic process in the foetus and, after birth, these infants are at high risk for anaemia. In particular, low reticulocyte counts at birth in infants treated with IUT were highly correlated with anaemia. In contrast, in infants without IUT treatment, high reticulocyte counts at birth, were strongly associated with anaemia and transfusion dependency which may indicate severe haemolysis despite no antenatal need of treatment.

If infants develop transfusion dependent anaemia after birth, the disease trajectory can extent to the first three months of life. However, to prevent unnecessarily long periods of follow-up and frequent blood sampling tests for haemoglobin levels, we showed with our large cohort of severe cases that follow-up can be closed at 45 days if there are no signs of anaemia and need for transfusion at that time for infants in good clinical condition with proper instruction to caregivers.

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