Optimising neonatal management of haemolytic disease of the foetus and newborn

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Chapter seven

Necrotising enterocolitis in haemolytic disease of the newborn: a retrospective cohort study

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Abstract

Background and objectives

Necrotising enterocolitis (NEC) is a common and often severe gastrointestinal emergency in newborn infants. While usually affecting (very) premature infants, an association between NEC and haemolytic disease of the foetus and newborn (HDFN) has been suggested. HDFN may be an additional risk factor to develop NEC. The objective of this study was to evaluate the occurrence of NEC in infants affected with moderate to severe HDFN in a large single centre cohort as compared to a broad population of infants without HDFN.

Materials and methods

Retrospective cohort study of medical records of neonates with and without HDFN, with a gestational age at birth ≥30 weeks and ≤38 weeks, and admitted to the Leiden University Medical Centre between January 2000 and December 2016.

Results

A total of 3284 patient records of infants born in the study period were reviewed and 317 cases of HDFN were identified. The incidence of NEC was significantly higher among infants with HDFN compared to infants without HDFN: 4/317 affected infants (1.3%) vs 11/2967 affected infants (0.4%, relative risk 3.40, 95% confidence interval 1.09-10.63).

Conclusions

We observed a higher incidence of NEC in an overall late preterm to near term population of infants with moderate to severe HDFN, compared to infants born without HDFN. The clinician taking care of an HDFN affected infant should be cautious of this higher risk.

Introduction

Necrotising enterocolitis (NEC) is one of the most common gastrointestinal emergencies in newborn infants and is defined by ischaemic necrosis of the intestine with a high mortality rate.[1] Epidemiologic studies have identified multiple risk factors for the development of NEC, such as prematurity and low birth weight.[2,3] NEC in late prematurity is rare, but could develop as a complication among otherwise predisposed infants.[4] This predisposition is likely an interplay of hypoxic-ischaemic injury of the gastrointestinal tract, physiological immaturity of the gastrointestinal tract and of the immune system, and alterations in the normal microbiological flora of the intestine.[5] Haemolytic disease of the foetus and newborn (HDFN) can influence peripheral oxygenation and may also influence the gastrointestinal system because of high bilirubin levels. Potential associations between NEC and HDFN or treatment for HDFN have been reported.[6,7]

Severe HDFN is a nowadays rare condition caused by an incompatibility between maternal and foetal red blood cell antigens. Maternal alloantibody formation against foetal red blood can cause foetal anaemia and, if left untreated, foetal hydrops and death.[8] Antenatal treatment in HDFN is mainly based on intrauterine erythrocyte transfusion (IUT) to the foetus in case of severe foetal anaemia, and in rare cases, early intravenous immunoglobulin (IVIg) treatment is started in pregnancy.[9] After birth, HDFN is characterised by hyperbilirubinaemia and ongoing anaemia, which can last up to three months of age.[10] Postnatal treatment of HDFN can include phototherapy, exchange transfusions, erythrocyte transfusions, and in some centres, IVIg is administered.

In the population of HDFN affected infants, several of the aforementioned predisposing factors for NEC are present. In the literature, both antenatal and postnatal treatments of HDFN have been associated with the occurrence of NEC.[11-13] Due to the rarity of severe HDFN and of NEC in late premature and near term infants, it is very difficult to conclusively investigate causative associations between HDFN, the treatment of HDFN, and the occurrence of NEC. It is therefore critical to report cases of NEC and HDFN to unravel the underlying pathophysiology of HDFN and NEC and their shared characteristics. The objective of this study was to evaluate and report the occurrence of NEC and various clinical characteristics in a large population of infants affected by HDFN, as compared to a broad population of infants admitted to the neonatal intensive care unit (NICU) of our centre without HDFN.

Methods

We conducted a retrospective cohort study of medical records of neonates with and without HDFN. The medical records of all live-born infants with a gestational age at birth ≥30 weeks and ≤38 weeks admitted to our NICU between January 2000 and December 2016 were reviewed for this study. Outborn patients and infants with major congenital abnormalities were excluded.

In the Netherlands, all pregnant women are routinely screened for the presence of alloantibodies in pregnancy and maternal blood samples with a positive screening result are sent to one of the two national referral laboratories (Sanquin Diagnostic Services or the Special Institute for Blood group Investigations (BIBO)). Thereafter, the clinical relevance of the antibody is evaluated by assessing the antibody specificity, and by assessing whether the foetus is antigen-positive. If the foetus is positive, the risk on foetal haemolysis is assessed by serially determining the antibody titre and antibody-dependent cell-mediated cytotoxicity (ADCC). The cut-off values for moderate to severe HDFN and referral to a specialised centre are set at an ADCC assay ≥50% in case of D immunisation and ≥30% in case of other blood group antigens, or antibody titres tested in maternal serum ≥1:16 in D and ≥1:2 in K immunisation.[14] These high-risk pregnancies are referred to the Leiden University Medical Centre (LUMC), the national referral centre for HDFN and intrauterine treatment in the Netherlands. At the LUMC, these 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 (MoM) velocity or if signs of hydrops are present, treatment with IUT is indicated. Planned delivery at the LUMC and neonatal admission to the NICU of the LUMC is recommended for all high-risk pregnancies.

The primary outcome was the relative risk to develop NEC in case of (moderate to severe) HDFN compared to a population of infants without HDFN delivered at a similar gestational age range. The characteristics of infants with HDFN and NEC were specified in more detail for full comprehension of these cases. *P*-values or other indicators of statistical significance were not reported as the various variables are not defined as outcome measures in this study, but have a descriptive nature.

NEC was defined according to Bell's criteria ≥ stage 2A and diagnosis was confirmed radiographically in the presence of pneumatosis intestinalis.[15] Several clinical characteristics that were derived from literature as potential predisposing factors for NEC were collected of this population. The following perinatal and neonatal data were collected: gender, gestational

age at birth, birth weight, small for gestational age (SGA) (defined as birth weight <10th centile for gestational age), multiple/singleton, mode of delivery, perinatal asphyxia, presence of umbilical or central venous catheter, NEC (≥2A according to Bell's criteria),[16] hypotension (treated with inotropics), proven sepsis (confirmed by a positive blood culture), need for ventilation, need for erythrocyte transfusion, need for exchange transfusion and mortality. In case of HDFN, the following additional data were collected: type of alloimmunisation, treatment with IUT (number of IUTs) and antenatal or postnatal treatment with IVIg.

The data were analysed using IBM SPSS Statistics (version 25.0; SPSS Inc, Chicago, IL). The primary outcome was reported as a relative risk with a 95% confidence interval. Other data are reported as n (%), mean or median (interquartile range [IQR] / standard deviation [SD]), depending on the underlying distribution of the data.

All data were collected as part of standard practice and were retrieved from the medical status from infants and mothers with written consent from parents and caregivers for infants with HDFN. For all (other) infants admitted to the NICU, parents and caregivers are informed of the use of medical data for retrospective research and can choose to opt out. The ethical committee of the LUMC reviewed the research proposal and confirmed the observational nature of the study and provided a waiver for further ethical consent.

Results

After excluding patients with major congenital abnormalities, the data of 3284 infants was included in the study. Of this population, 317 (9.7%) infants were admitted for HDFN and were compared to infants without HDFN (n=2967).

Table 1 shows the baseline characteristics of the cohort, infants admitted with HDFN were more often male (61.5%) and had a median gestational age at birth of 36 weeks (IQR 36-37). Infants without HDFN had a median gestational age of 34 weeks (IQR 31-36 weeks). Infants with HDFN had, in accordance with the higher median gestational age at birth, a higher median birth weight of 2900 grams (IQR 2610-3203) than unaffected infants, which had a median birth weight of 2222 grams (IQR 1640-2715). Infants with HDFN had also lesser occurrence of being born SGA (2.5 vs 12%) and were more often singletons (97.4 vs 39.7%) compared to infants without HDFN.

Table 1. Baseline characteristics

	HDFN (n = 317)	No HDFN (n = 2967)
Male - n (%)	195 (61.5)	1543 (52.0)
Caesarean delivery - n (%)	83 (26.2)	1268 (42.7)
Gestational age at birth (weeks) - median (IQR)	36 (36-37)	34 (31-36)
Birth weight (grams) - median (IQR)	2900 (2610-3203)	2222 (1640-2715)
SGA - n (%)	8 (2.5)	356 (12.0)
Singleton - n (%)	309 (97.4)	1178 (39.7)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *n*, number; *SGA*, small for gestational age.

During the study period, there were 4 (1.3%) infants with NEC in the HFDN population and 11 infants (0.4%) with NEC in the population without HDFN of similar gestational age (Table 2). The relative risk of developing NEC is therefore 3.40 (1.3/0.4; 95% confidence interval 1.09-10.63). Clinical characteristics with a potential association with NEC varied among HDFN-affected and HDFN-unaffected infants and are shown in Table 2. Umbilical catheters, erythrocyte transfusions and exchange transfusions were more present among infants affected by HDFN.

Table 2. Clinical outcomes

	HDFN (n = 317)	No HDFN (n = 2967)
NEC - n (%)	4 (1.3)	11 (0.4)
Need for ventilation - n (%)	6 (1.9)	260 (8.8)
Hypotension requiring inotropics - n (%)	3 (0.9)	66 (2.2)
Umbilical catheter - n (%)	103 (32.5)	482 (16.2)
Central venous catheter - n (%)	14 (4.4)	187 (6.3)
Erythrocyte transfusion - n (%)	76 (24.0)	155 (5.2)
Exchange transfusion	52 (16.4)	2 (0.1)
Proven sepsis - n (%)	14 (4.4)	140 (4.7)
Perinatal asphyxia - n (%)	1 (0.3)	34 (1.1)
Mortality - n (%)	2 (0.6)	15 (0.5)

HDFN, haemolytic disease of the foetus and newborn; *n*, number; *NEC*, necrotising enterocolitis.

When comparing infants with NEC and HDFN to infants with NEC without HDFN, NEC occurred at a higher median gestational age at birth and higher birth weight among HDFN-affected

infants compared to infants that suffered from NEC without HDFN (34 vs 31 weeks and 1879 vs 1201 grams; respectively, Table 3). A significant portion of infants with NEC without HDFN was born SGA compared to infants with NEC and HDFN (45.5 vs 0.0%). Clinical features between affected infants with and without HDFN are shown in Table 3.

Table 3. Characteristics of NEC cases

	NEC HDFN (n = 4)	**NEC no HDFN (n = 11)**
Male - n (%)	1 (25.0)	4 (36.3)
Caesarean delivery - n (%)	3 (75.0)	8 (72.7)
Gestational age at birth (weeks) - median (IQR)	34 (31-35)	31 (30-33)
Birth weight (grams) - median (IQR)	1879 (1289-2052)	1201 (807-1606)
SGA - n (%)	0 (0.0)	5 (45.5)
Need for mechanical ventilation - n (%)	1 (25.0)	4 (36.3)
Hypotension - n (%)	0 (0.0)	2 (18.1)
Umbilical catheter - n (%)	2 (50.0)	7 (63.4)
Central venous catheter - n (%)	3 (75.0)	7 (63.4)
Erythrocyte transfusion - n (%)	2 (50.0)	3 (27.3)
Exchange transfusion - n (%)	1 (25.0)	0 (0.0)
Proven sepsis - n (%)	0 (0.0)	3 (27.5)
Perinatal asphyxia - n (%)	0 (0.0)	0 (0.0)
Mortality - n (%)	1 (25.0)	2 (18.1)

HDFN, haemolytic disease of the foetus and newborn; *IQR*, interquartile range; *n*, number; *NEC*, necrotising enterocolitis*; SGA*, small for gestational age.

Of the infants with HDFN, four infants developed NEC, which are specified in Table 4. In the entire HDFN population of this study, the majority was diagnosed with D immunisation (75.4%) and treated with IUT (64.4%). A total of 52 patients (16.4%) needed exchange transfusion. Postnatal erythrocyte transfusions were given to 76 (24.0%) patients during the initial admission to the LUMC after birth. Compared to the overall HFDN population, the infants that developed NEC had a lower gestational age (range 30-35 weeks, compared to a median of 36 weeks) and lower birth weight (range 1147-2055 gram, compared to a median of 2900 gram). Of the four cases, there were two cases of D alloimmunisation, one of K immunisation and one of c immunisation. All affected infants were treated with IUT, varying between a total number of 1-5 IUTs. One infant was treated with antenatal IVIg treatment, one received

exchange transfusion after birth (before the onset of NEC) and two infants received erythrocyte transfusions after birth (after the onset of NEC). Three infants were treated conservatively and survived, one required surgery for NEC and did not survive.

Table 4. NEC in HDFN population

	CASE NO. 1	**CASE NO. 2**	**CASE NO. 3**	**CASE NO. 4**	**HDFN population** (n=317)
Gender	Male	Female	Female	Female	Male 195 (61.5)[a]
Gestational age at birth (weeks)	33	34	30	35	36 (36-37)[b]
Birth weight (grams)	2043	1715	1147	2055	2900 (2610-3203)[b]
Type of alloimmunisation	D	D	c	K	239 (75.4) D, 41 (12.9) K, 37 (11.7) other
Treated with IUT	Yes	Yes	Yes	Yes	204 (64.4)[a]
Number of IUTs	5	2	1	5	2 (0-3)[b]
Antenatal IVIg treatment	No	No	No	Yes	9 (2.8)[a]
Postnatal IVIg treatment	No	No	No	No	41 (12.9)[a]
Haemoglobin level at birth (g/dL)	9.0	9.5	19.7	13.9	13.1 ± 3.4[c]
Erythrocyte transfusion	Yes	Yes	No	No	76 (24.0)[a]
Exchange transfusion	Yes	No	No	No	52 (16.4)[a]
Onset of NEC (day of life)	4	3	10	5	-
Treatment of NEC	Conservative	Conservative	Conservative	Surgical	-
Survival	Yes	Yes	Yes	No	315 (99.4)[a]

HDFN, haemolytic disease of the foetus and newborn; *IUT*, intrauterine transfusion; *IVIg*, intravenous immunoglobulin; *NEC*, necrotising enterocolitis.
[a] Number (%); [b] median (interquartile range); [c] mean ± standard deviation.

Discussion

The objective of this study was to evaluate and compare the occurrence of NEC in infants affected by HDFN to infants admitted to the NICU of our centre without HDFN. During the 17-year study period, we found a relative risk to develop NEC for infants with HDFN of 3.40 (95%-confidence interval 1.09-10.63), although the absolute risk of developing NEC in our HDFN cohort was low (1.3%). These findings are consistent with previous reports suggesting HDFN as an additional predisposing factor for NEC.[7]

We report on a cohort of moderate to severe HDFN, as defined by antibody titres and ADCC values, as mild HDFN is no indication for referral to the NICU of the LUMC. Cases of mild HDFN can only be expected at our NICU as a secondary (or tertiary) diagnosis, and in the data of infants without HDFN may therefore be under reported. However, mild HDFN with a low ADCC and/or antibody titre and no need for intrauterine monitoring or transfusions, or intensive phototherapy, is unlikely to bias the potential relation with NEC.

We acknowledge that the infants admitted to the NICU without HDFN are a reference group with a broad range of pathology and treatments, but despite the great heterogeneity in the population without HDFN, a higher occurrence of NEC was found in HDFN affected infants. Despite the lower occurrence of NEC among infants without HDFN, known risk factors for NEC were actually more present, including a lower median gestational age at birth, lower median birth weight and these infants were more often SGA at birth.

Furthermore, while we report on a very large sample of HDFN, we acknowledge the low incidence of NEC in this study population and are cautious to draw conclusions with merely 15 infants with NEC in our study distributed over a long time period. This low occurrence rate of NEC in our study prevents reliable risk factor analysis and perhaps the study should, in terms of accuracy, be considered as an extended cases series. However, for hypothesis-generating purposes, the data were carefully further explored. One can speculate that the higher risk to develop NEC might be due to the relative hypoxia as part of the pathophysiology of HDFN itself, but NEC has also been suggested to be a complication of IUT or (postnatal) IVIg treatment.[6] These suggested associations are complicated as, for example, IUT and IVIg treatment may have a treatment-related effect on the foetal circulation and oxidative state, but are in itself also signs of more severe HDFN and thus anaemia, which is also associated with NEC.[17] The high occurrence of erythrocyte (exchange) transfusions in the infants with HDFN could similarly be seen as potential predisposing factor for NEC, but is also a reflection of the severity of HDFN. More severely affected HDFN infants are in higher need of phototherapy and exchange transfusions (hence the higher need for umbilical catheters) and erythrocyte transfusions.[10] In our population of HDFN-affected infants, all four infants that developed NEC received IUTs and only one was treated with antenatal IVIg. Two of the four affected infants received a strikingly high number of five IUTs, while only one infant required an exchange transfusion. While the infants in this study with HDFN were actually born at a median higher gestational age and higher median birth weight compared to the infants without HDFN, the four infants that developed NEC had lower gestational ages and lower birth weights compared to the other HDFN infants. This might carefully imply that the effect of prematurity and low birth

weight to develop NEC is enhanced in the presence of one or more additional predisposing factor, such as (treatment for) HDFN.

HDFN is a rare disease and due to better and more preventive measures and treatment options, the number of affected patients is small and still declining, which complicates extended research within this population. However, our centre is the national referral centre for HDFN in the Netherlands and as a result a substantial database of HDFN patients has been created, in which we try to observe and investigate rare outcomes. Our results emphasise that the HDFN population should be concerned as a distinct entity of the NICU population, with potentially distinct risk factors contributing to the development of NEC. The clinician taking care of an HDFN-affected infant should be cautious of this rare but serious complication and perhaps especially in otherwise predisposed infants by late prematurity or a low birth weight.

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