Risk factors and outcomes of NEC in preterm and low birth weight infants with a congenital heart defect

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Abstract

Background and aims: Risk factors for necrotizing enterocolitis (NEC) in preterm and low birth weight (LBW) infants with a congenital heart disease (CHD) are unclear and inconsistent. This study aimed to identify the risk factors and outcomes of NEC in preterm and low birth weight infants born with a congenital heart disease in the population of the UMCU.
Study design/methods: Single-center retrospective cohort study of 62 preterm (<35 weeks gestational age (GA)) and LBW (<2500g) infants with a congenital heart disease (CHD) over an 8-year period from January 2008 to December 2019, admitted to the NICU or PICU of the Wilhelmina Children's hospital. Patients were grouped in a NEC and no-NEC group. Analyses of clinical characteristics, factors and pre- and postoperative variables between the NEC and no-NEC group were conducted. Only univariable analysis was performed due to small sample size.
Results: In this study population of 62 preterm and LBW infants with a CHD, 14 developed NEC (22.6%). The well-known risk factors for NEC, gestational weight at birth (p=0.28), birth weight (p=0.46) and feeding (p=0.13) were not associated with NEC in this population. NEC was more common in infants that received invasive ventilation (54% vs 92.9%, p= 0.011) and CPR preoperatively (median of 2 vs 10, p=0.33). Infants that developed a sepsis also had a higher incidence of NEC (45.8% vs 78.6%, p= 0.037). All infants received invasive ventilation postoperatively. The duration of invasive ventilation post operation was higher in infants that developed NEC (median of 3.25 vs 4, p= 0.07). Infants with signs of PHT (16.7% vs 46.2%, p=0.038) or iNO treatment (6.3% vs 38.5%, p=0.008) were also more likely to develop NEC.
Conclusion: The incidence of NEC in this more vulnerable population was substantial and the infants with NEC had a higher mortality rate. This confirms the high-risk of current population. Variables that were associated with an elevated risk of NEV were: invasive ventilation preoperatively, received CPR preoperatively, sepsis preoperatively, duration of 1st invasive ventilation postoperatively, signs of PHT on ultrasound and iNO treatment. Results should be carefully interpreted due to small sample size.

1. Introduction

Necrotizing Enterocolitis (NEC) remains a severe gastrointestinal disorder in the neonatal period leading to high mortality and morbidity. Most cases of NEC occur in preterm and low birth weight (LBW) infants, research shows that the prevalence varies from 5 to 10% in this population.[1] The clinical outcomes of NEC in these infants have fortunately improved in the past years due to advances in technology, earlier recognition and more aggressive treatment, but mortality rates ranging from 15% to 30% remain substantial.[2,3] This is caused by challenges in the management most likely as result of the incompletely understood multifactorial pathogenesis.[1] NEC susceptibility in preterm and LBW infants can be, to some extent, explained by inherent intestinal and immunological immaturity.[4] This agrees with consistent reports that low gestational age and LBW, representatives of immaturity, are clear risk factors.[5] An additional major risk factor is the presence of a congenital heart disease (CHD). The population of preterm infants with CHD has grown in the past years this is a result of the overall improved survival of preterm infants due to advances in technology and therapy.[6] However, care of these preterm infants with CHD unfortunately stays complicated. No consensus on the form of therapy, time of surgery, intervention type and prevention of complications complicate the care of preterm infants with CHD.[7] CHD in term infants raises the risk for NEC by at least 10-fold and CHD in preterm/LBW infants raised the risk up to 100-fold compared to the general population of newborns.[8] This because the pathophysiology of NEC for infants with a CHD differs from the pathophysiology for preterm and LBW infants. The first

thought to mostly stem from mesenteric hypoperfusion caused by a cardiac disease.[9,10] Evidence thus suggests that these hemodynamic changes caused by CHDs have an additive effect on the risk for NEC in preterm and or LBW infants.[11] Preterm and LBW infants with CHD are therefore more vulnerable for NEC. [8,12] Many risk factors for NEC have been identified, however experts disagree about their value and significance. The only factors there was high agreement on by experts were gestational age, birth weight and feeding.[5,13] Aside from these factors, a systemic review of high and moderate quality studies reported that the following characteristics were also significant prognostic factors in at least 2 of the included studies; ethnicity, being outborn, C-section, hypotension, sepsis, and surfactant therapy. [2] This review also concluded that high quality studies on risk or prognostic factors for NEC are rare and included studies showed inconsistent results among possible risk factors.[2,5] Risk/prognostic factors thus remain unclear, this leads to uncertainty in determining patients at risk. This study primary objective was to examine which variables are risk factors for NEC. Secondary measures included the prevalence of NEC, 1-year mortality for all infants with NEC and 1-year mortality for infants with either NEC stage I, II, III.

2. Methodology

Design

This was a retrospective cohort study of all preterm (<35 weeks gestational age (GA)) and LBW (<2500g) infants with a CHD requiring therapeutic cardiac intervention (either therapeutic cardiac surgery or therapeutic heart catheterization) within the first year of life, from the NICU and PICU of the Wilhelmina Children's Hospital Utrecht, born between January 2008 and December 2020.
The primary objective was to examine which variables are risk factors for NEC. Secondary measures included the prevalence of NEC, 1-year mortality for all infants with NEC and 1-year mortality for infants with either NEC stage I, II or III.

Data acquisition

Data was obtained from electronic patient record systems HIX and Meta-Vision. This study only included LBW (<2500gr) infants with CHD under 35 weeks of gestational age. This differed from the common classification of preterm infants (<37 weeks of gestational age). These near-term infants born between 35- and 37-weeks GA were excluded because they have a much lower incidence and lower risk for NEC compared to infants under 35 weeks of GA.[14] We excluded infants who had an isolated patent ductus arteriosus and who did not have an intention to treat approach within the first year of life due to CHD, pre – or dysmaturity or any other genetic/ extracardiac malformation. Infants were classified in a group that developed NEC and a group that did not. The diagnosis NEC was defined as Bell's stage 2 or higher, this to solely include infants with proven NEC in the NEC group and to exclude infants with presumed or suspected NEC.
Maternal and neonatal variables, feeding data, surgical variables and pre- and postoperative data were collected and compared between the NEC group and no-NEC group. The selection of these variables was based on previous evidence, expert opinion, or potential correlation to NEC. NEC was diagnosed and staged using the modified Bell's criteria.[15] Maternal and neonatal variables mostly consisted of demographic characteristics and classifications of the CHDs. The CHDs were anatomically categorized using the Clancy classification. [16] CHDs were also categorized, using expert knowledge, into cyanotic and

non-cyanotic CHDs. Both carried out by a master medical student and supervised by an expert pediatric intensivist. Feeding variables included type of feeding, age at start of enteral feeding, feeding continuity, use of fortifiers, use of acid suppression and whether feeding goal for preterm infants was reached. The latter variable was defined as reaching enteral feeding goal pre-intervention based on the local feeding protocol. Pre- and postoperative variable definitions are listed in appendix A.

Ethical considerations

This study was part of a larger ongoing project carried out by a scientific research institute of the UMCU. The study was conducted in accordance with EU GDPR. Informed consent was not required due to the patient numbers and the retrospective nature of the study according to the first exemption of the Code of conduct for medical research.

Statistical analysis

Statistical analysis was performed using SPSS. Differences between the NEC and no-NEC group were assessed using Chi-square tests when variables were dichotomous. Continuous variables were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov test. All continuous variables were non-normally distributed and assessed accordingly using Mann-Whitney U tests. Multiple regression analysis was not possible due to a small sample size and small number of observations. Correction for multiple testing was well-considered, given the stringency of the correction methods we decided not to perform this, but took into account the risk of false positive results. The study results were carefully considered and compared to previous studies.

3. Results

During the study period a total of 62 infants that met our inclusion criteria were admitted to the NICU and PICU of the Wilhelmina Children's Hospital Utrecht. 14 of these infants developed NEC and were grouped in the NEC group (figure 1). Categorization using the modified Bell's criteria divided the 14 NEC in 2 patients with stage 1b NEC, 9 patients with stage 2a NEC, 2 with stage 2b NEC and 1 with stage 3a NEC. The median age at diagnosis was 17.5 (IQR [6.5, 24.25]) days, 13 (93%) of the infants developed NEC preoperatively.

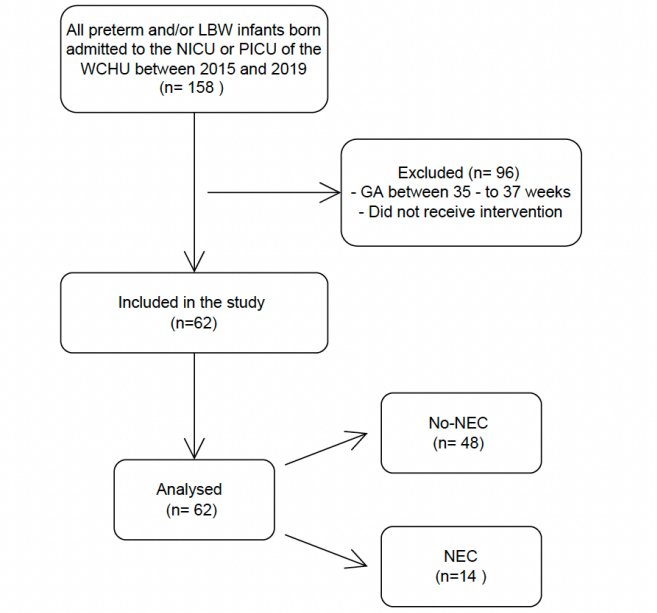

Figure 1. Flow chart of study participants

The most common cardiac diagnosis in all the patients was ventricular septal defect (VSD) and the primary cardiac diagnosis of the NEC patients included VSD as well. The categories of these cardiac diagnoses based on the Clancy risk score and division in cyanotic and non-cyanotic disease are illustrated in table 1. Most NEC patients had a Clancy risk score of 1. And the incidence of NEC among the cyanotic patients was 7 (50%).

Table 1 shows the neonatal characteristics between the NEC and No-NEC group. No statistically significant differences in the neonatal characteristics were found between the two groups (P>0.05). It should be mentioned that the NEC group notably had more male infants than female infants compared to the no-NEC group. The cause of this difference in gender is unknown, previous studies have abolished an association between gender and mortality.[5] Furthermore, the median [IQR] gestational ages for the NEC group and no-NEC group were 32.86 [31.29 – 34.00] weeks and 31.86 [30.29 – 33.71] weeks, respectively. The median [IQR] birth weight in NEC group (1405 grams [1075, 2022]) was notably lower than the median [IQR] no-NEC group (1652 grams [1222, 2151]), but also did not differ statistically.

Table 1 Neonatal characteristics of subjects with NEC and without NEC

Neonatal Characteristics	No NEC n = 48	NEC n = 14	P-value
Gender: n/N (%) Male Female	24/48 (50) 24/48 (50)	10/14 (71.4) 4/14 (28.6)	.266
Median gestational age [IQR], days	32.86 [31.29, 34.00]	31.86 [30.29, 33.71]	.345
Classification of prematurity (week): n/N (%) <28 28 to <32 32 to <34 34 to ≤35	 4/48 (8.3) 13/48 (27.1) 17/48 (35.4) 14/48 (19.1)	 0 (0) 7/14 (50) 4/14 (28.6) 3/14 (21.4)	.450
Apgar score 1-min: median [IQR] 5-min: median [IQR]	 7 [5-8] 7 [1-7]	 8 [7-9] 7 [6-9]	 .176 .425
Median umbilical cord pH [IQR], pH	7.26 [7.22, 7.31]	7.34 [7.23, 7.72]	.115
Median birth weight [IQR], grams	1652 [1222, 2151]	1405 [1075, 2022]	.466
Birth weight classification: n(%) > 2500; ≤ 2500; ≤ 1500; ≤ 1000	 4/48 (8.3) 22/48 (45.8) 17/48 (35.4) 5/48 (10.4)	 0 5/14 (35.7) 7/14 (50) 2 /14 (14.3)	.540
Head circumference at birth(cm): median [IQR]	29.25 [27, 31]	29 [26, 30]	.198
Clancy score I II III	 33/48 (68.8) 13/48 (27.1) 2/48 (4.2)	 9/14 (64.3) 4/14 (28.6) 1/14 (7.1)	.887
Cyanotic CHD disease: n/N (%)	14/48(29.9)	7/14 (50)	.201
Definitive intervention delayed with provisional intervention: n/N (%)	11/48 (22.9)	5/13 (38.5)[#]	.297
Prenatally diagnosed CHD: n/N (%)	23/48 (47.9)	10/14 (71.4)	.121
Born in tertiary center: n/N (%)	11/48 (22.9)	4/14(28.6)	.664
Cause associated anomalies: n/N (%)	8/48 (16.7)	2/14 (14.3)	.602
Cause associated anomalies with genetic diagnosis: n(%)	5/8 (62.5)	2/2 (100)	.585

Abbreviations: CHD, congenital heart disease

No neonatal variables were risk factors in this study

[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was lower than the true total of patients

No maternal characteristics showed a statistical association (P>0.05) with NEC in this population (Table 2). Maternal hypertension and administration of steroids antenatally were relatively more common for the infants that developed NEC but did not reach statistical significance.

Table 2 Maternal characteristics of subject with NEC and without NEC

Maternal characteristics	NO NEC n = 48	NEC n = 14	P-value
Maternal age (years): median [IQR]	31[29, 34]	33 [29.25, 35]	.535
Multiparity: n/N (%)	22/48 (45.8)	6/14 (42.9)	.214
Number of previous pregnancies: median [IQR]	2 [1, 2]	3 [1, 5]	.389
Maternal HT: n/N (%)	5/48 (10.4)	3/14 (21.4)	.478
Antenatal steroids: n/N (%)	17/48 (35.4)	7/14 (50)	.308
Induced labor: n/N (%)	32/48(66.7)	8/14 (57.1)	.512
Indication for induced labor: n/N (%) Maternal Fetal	 11/32 (34.4)[#] 20/32 (62.5)[#]	 0 8/8 (100)[#]	.117
Obstetric infection: n/N (%)	4/48 (8.3)	0	.380

Abbreviations: HT, Hypertension defined at systole > 130 and diastole > 80
No maternal variables were risk factors in this study
[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was lower than the true total of patients

Table 3 shows the difference in feeding patterns between the NEC and no-NEC group. We did not find any statistically significant feeding differences between the two groups. The NEC group relatively contained more infants (5/12 (41.7%)) that received preterm formula exclusively, compared the no-NEC group (7/46 (15.2%)). Both groups had comparable start dates of enteral feeding, thus NEC infants did not have a delayed start in feeding.

Table 3 Feeding patterns of subjects with NEC and without NEC

Feeding	No NEC N = 48	NEC N = 14	P value
Type of feeding: n/N (%) - Breast - Preterm formula - Combination of above	 23/46 (50) 7/46 (15.2) 12/46 (26.1)	 6/12 (50) 5/12 (41.7) 1/12 (8.3)	.133
Age at start of enteral feeding(days): median [IQR]	1 [1-2]	1[1-2]	.677
Feeding continuity: n/N (%) - Continuous - Intermitted bolus - Combination of above	 0 42/46 (91.3) 3/46 (6.5)	 0 10/12 (83.3) 2/12(16.7)	.627
Reached feeding goal for preterm infants before intervention: n/N (%)	17/43 (39.5)	3/12 (25)	.432
Use of fortifiers: n/N (%)	31/47 (66)	7/13 (53.8)	.480
Use of acid suppressors: n/N (%)	3/47 (6.4)	2/12 (16.7)	.418

[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was frequently lower than the true total of patients.

The type of surgery and timing were not associated with NEC in this population (Table 4). The median weight at intervention differed between the groups, with a median [IQR] weight of 2520 grams [1945.5, 3385] and 2335 [2177, 2850] for no NEC and NEC infants respectively, but did not differ statistically (p>0,05).

Table 4 Surgical characteristics of subjects with and without NEC

Surgical characteristics	No NEC n = 48	NEC n = 14	P value
Type of first intervention			
- Catheterization	19/48 (39.6)	5/13 (38.5)[#]	.941
- Surgery	29/48 (60.4)	8/13 (61.5)[#]	
Age at surgery			
- Chronological age(days): median [IQR]	42 [11, 103]	36 [22.5, 64]	.937
- PMA: median [IQR]	37.93 [33.4, 47,1]	37.57 [36.5, 40]	.647
Weight at intervention (grams): median [IQR]	2520 [1945.5, 3885]	2335 [2177, 2850]	.337

Abbreviations: PMA, post menstrual age
No surgical characteristics were associated with NEC in this study
[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was lower than the true total of patients

Preoperative factors associated with an increased risk of NEC in this population included invasive ventilation, CPR, and sepsis (Table 5). No other analyzed preoperative factors were found to be significant. More preoperative variables, in addition to table 5, were analyzed in this study. For improved readability we reported the most common variables and those with the lowest p values in the results and enumerated the full list of variables in appendix B.

All infants received mechanical ventilation postoperatively (Table 6). The duration of invasive ventilation postoperatively differed significantly ($p < 0.05$) between the groups. Other variables that differed between the no - NEC and NEC group were signs of pulmonary hypertension (PHT) on ultrasound and inhaled Nitric Oxide (iNO) treatment. No other analyzed postoperative factors were found to be significant. See **appendix B** for the full list of analyzed variables.

Survival in NEC infants

The mortality rate differed significantly between no NEC infants and NEC infants (respectively, 6 out of 48 vs 6 out of 14; P 0.04). All deaths in infants without NEC were directly attributable to the underlying heart condition while 4 out of the 6 deaths in infants with NEC were attributable to NEC or combination of complications such as sepsis, poor prognosis, and initiation of palliative care.

As secondary measure we also investigated the mortality per Bell's criterium. 1 of 2 patients with Bell's 1a died (50%), idem ditto for bells 2a (1 of 2 patients died, 50%), 2 of 7 patients with Bell's 2a died (29%), both 2 patients with Bell's 2b, and the only patient with Bell's 3a also died.

Table 5

Preoperative variables	No NEC n =48	NEC n =14	P value
Airway			
Invasive ventilation: n/N (%)	26/48 (54.2)	13/14 (92.9)	**.008**
Duration of 1st invasive ventilation (days): median[IQR]	4 [2, 7]	6 [1.5, 16]	.362
Non-invasive ventilation; n/N (%)	33/48 (68.8)	10/14 (71.4)	.74
Duration of non-invasive ventilation (days): median[IQR]	1.5 [0.5, 12.5]	7.5 [.88, 13.25]	.353
Circulation			
Received CPR: n/N (%)	1/48 (2.1)	3/14 (21.4)	**.010**
CPR duration(days): median [IQR]	2 [2, 2]	10 [10, 10]	.083
Age at CPR*			
- Chronological age (days): median [IQR]	68 [68, 68]	74 (0, -)*	.655
- PMA: median [IQR]	39.85 [39, 39]	43.62[32.3, -]	.655
Arrhythmias: n/N (%)	1/48 (2.1)	1/14 (7.1)	.346
Intubation due to circulatory failure: n/N (%)	14/48 (29.2)	3/14 (21.4)	.568
Pulmonary hypertension: n/N (%)	9/48 (18.8)	4/14 (28.6)	.646
Sepsis: n/N (%)	22/48 (45.8)	11/14 (78.6)	**.031**

Abbreviations: CPR, cardiopulmonary resuscitation; PMA, post menstrual age

* 4 infants received CPR of which 3 in the NEC group. The age of 1 infant in the NEC group was unknown. This is illustrated by the hyphen "-"

Table 6

Postoperative variables	No NEC n = 48	NEC n = 14	P value
Airway			
Invasive ventilation: n/N (%)	48 (100)	13/13 (100)[#]	-
Duration of 1st invasive ventilation(days): median [IQR]	3.25 [1, 6]	4 [2, 16.5]	**0.07**
Non-invasive ventilation: n/N (%)	25/47 (53.1)[#]	9/13 (69.2)[#]	.508
Duration of non-invasive ventilation (days): median [IQR]	2 [0.75, 6]	4 [1.75, 28.75]	.148
Circulation			
Received CPR: n/N (%)	3/48 (6.3)	2/13 (15.4)[#]	.287
CPR duration(days): median [IQR}*	2[2,2]	5 [5, 5]	.317
Number of CPR cycles(n): median[IQR][+]	0	2 [2, 2]	-
Age at CPR			
- Chronological age (days): median[IQR]	80 [0, -]	129 [72, -]	1
- PMA: median[IQR]	38,1 [33, -]	51,2 [41.4, -]	.564
Arrhythmias: n/N (%)	3/48 (6.3)	3/13 (23.1)[#]	.071
LCOS			
- Inotropics 48h postoperatively (incl. milrinon): n/N (%)	22 (45.8)	7/13 (53.8)	.701
- 48h postop lactate >3: n/N (%)	6 (12.5)	3/13(23.1)	.504
- 48h postop: urine output <1ml/kg/h (6hours): n/N (%)	14 (29.2)	4/13 (30.8)	.756
48h postop ECLS: n/N (%)	0	0	-
Signs of PHT on ultrasound: n/N (%)	8 (16.7)	6/13 (46.2)	**.038**
iNO treatment: n/N (%)	3 (6.3)	5/13 (38.5)	**.002**

Abbreviations: CPR, cardiopulmonary resuscitations; PMA, post menstrual age; LCOS, low cardiac output syndrome; PHT, pulmonary hypertension.

[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was lower than the true total of patients

* Duration of CPR was only known for 2 infants, one in both the no NEC and NEC group.

[+] Number of CPR cycles was missing for the infants in the no NEC group and known for 1 infant in the NEC group.

4. Discussion

Many previous studies have researched NEC in preterm/LBW infants or NEC in infants with a CHD. These studies have either demonstrated a clear relationship between the age or birth weight of infants and NEC, and a clear relationship between CHD and NEC. The studies also showed that the pathophysiology of NEC differs when comparing NEC in preterm and LBW infants and NEC in infants with a CHD. Studies that investigate the more vulnerable population of preterm and LBW infants who also have a CHD are scarce, making comparisons in this discussion challenging.

This study combined the before mentioned characteristics to investigate risk factors of NEC **i**n preterm and LBW infants with a CHD. We found that the incidence of NEC among the 62 preterm and LBW infants with CHD was 22,6%, this is higher than the rate of 3 per 1000 newborns in the whole infant population and higher than the global incidence of 2-13%in preterm and low LBW infants.[17–19] While the incidence was not the focus of this study, it is worth mentioning that the high incidence in this study (compared to the global incidence of preterm and LBW) is consistent with the belief that CHD is a predisposing condition that can raise the risk by at least 10-fold.[8] The high incidence in this study can also be explained by the more vulnerable population of preterm (<35 weeks GA) and LBW infants with CHD.

This study design focused on determining whether specific risk factors for NEC could be identified in a population of preterm and LBW infants with a CHD. Many risk factors have been identified in several studies however experts disagree about their value and significance. The only factors there was high agreement on were GA, BW and feeding.[5,13] While decreasing GA and birth weight are consistently reported to be risk factors for NEC, this study did not find an association between GA or BW and NEC. Considering the strong evidence in previous studies this non-significance is most likely the cause of this studies small sample size. In previous studies, breast feeding has also consistently shown to decrease the risk of NEC and preterm formula to increase the risk of NEC.[20] This stems from the hypothesis that partially digested formula can provide a substrate for bacterial proliferation.[13] In this population, type of feeding was also not associated with high risk for NEC, probably also attributable to the small sample size. Nevertheless, preterm formula feeding was more common in the NEC infants of this study population. When considering that maternal breast milk production can be delayed in preterm birth, preterm formula feeding could be corrected for gestational age for accurate results.

The different surgical characteristics in this study did not show any statistical association with NEC. The age and weight at first intervention in the NEC infants were somewhat lower compared to the no-NEC infants. This could be explained by the inherent poorer characteristics and prognosis of NEC infants. But this may be misleading considering we did not find statistically significant associations and we could not compare this to previous studies, as there were none.

We reported that invasive ventilation was associated with an increased risk of NEC. This coincided with the findings of the systematic review of Samuels et al. that studied assisted ventilation.[2] However, a more recent review did not find consisted association between mechanical ventilation and NEC.[5] This raised the question whether ventilation has a causal relationship with NEC or is only associated, the latter reflecting that the sickest patients (with the highest risk for NEC) need more ventilation.

3 of the 4 infants that received CPR in this population developed NEC, leading to a statically significant association between CPR and NEC. Sepsis was also more common in the NEC group compared to the non-NEC group with an incidence of 11(78.6%,) and 22 (45.8%) respectively, p value <.05. No previous studies were done to compare these results.

All infants in this study population received invasive ventilation postoperatively. The duration of invasive ventilation differed significantly between the no-NEC and NEC group. Other postoperative variables that were significantly associated with NEC in this population were PHT on ultrasound and iNO treatment.

There were no other preceding studies that investigated the association between these variables and NEC. iNO is a treatment for respiratory failure in newborn infants that have a persistent high pulmonary vascular pressure. The role of iNO treatment for infants <35 weeks gestational age is not yet clear, but research shows improved outcomes in infants $\geq$ 35 weeks of gestational age.[21] This demonstrates that the association between iNO treatment and NEC only reflects higher frequency of iNO treatment in sicker patients who have an inherent higher risk for NEC.

Limitations

The main limitations of this study are the retrospective nature and small monocenter population size. No multivariable analysis was performed due to small sample size, and we did not correct of multiple testing. Therefore, the results should be carefully interpreted. A strength of this study was the comparability between the included cases. All cases in this study were treated with same consistency of the hospitals department. This made treatment and diagnostic regimens similar and comparable.

5. Conclusion

In this study population of 64 preterm and LBW infants with CHD, the incidence of NEC was substantially higher than the general population. This incidence coincides with the experience that prematurity, LBW and CHD increase the risk for NEC up to 100- fold. This study population exclusively consisted of vulnerable preterm infants under 35 weeks of GA which explains the high incidence of NEC in this population.

The predisposing conditions for NEC in this population of preterm and LBW infants with CHD were invasive ventilation, received CPR and sepsis preoperatively, and duration of 1st invasive ventilation, PHT and iNO treatment postoperatively. Factors with noticeable difference between the NEC and no-NEC group were gestational age, birth weight, type of feeding and surgical characteristics. These results should be carefully interpreted due to lack of power.

As expected, NEC significantly increased the risk of mortality in the infants of this study population, the deaths directly or indirectly attributed to NEC or its complications.

This study ads to the understanding of the ambiguity of many different variables. The results of this study will hopefully support future risk factor studies, act as a basis for prospective and multicenter studies and will hopefully add to future preventive strategies.

Appendix

A. Definitions

Variable	Definition
Neonatal and maternal variables	
Risk classification CHD (Clancy score)	Preoperative risk- of- death prediction model: Class I: two-ventricle heart without arch obstruction Class II: two-ventricle heart with arch obstruction Class III: single ventricle heart without arch obstruction Class IV: single ventricle heart with arch obstruction
Cyanotic CHD disease	Heart disease that results in reduced delivery of oxygen to the body
Born in tertiary center	Highly specialized medical care center, these were: - Wilhelmina Children's Hospital Utrecht - Center of congenital cardiac surgery: Rotterdam, Leiden/Amsterdam, Groningen - Other perinatology center in the Netherlands: Veldhoven, Maastricht, Nijmegen, or Zwolle
Associated anomalies	Other anomalies that were associated with the genetic disorder that caused the congenital heart disease (CHD) Unknown: If there was no genetic screening
Antenatal steroids	Initial admission of steroids at least 48 hours before delivery
Obstetric infection	Any viral or bacterial infection during the obstetric period that may cause complication for the mother or unborn child
Feeding variables	
Type of feeding	We categorized three types of feeding.: - Breast feeding - Preterm formula - Combination of feeding
Reached feeding goal for preterm infants before intervention	Reaching enteral feeding goal pre-intervention based on the local feeding protocol
Use of fortifiers	Protein and mineral supplement concentration added for extra nutrition. Examples are: - Breast milk fortifier (BMF) - Neonatal protein fortifier (NPF)/ Protifar Calogen, Solagen, Fantomalt, Friso 1 premature (F1P) were not recognized as fortifiers
Pre- and postoperative variables	
Surgery delaying cardiac catheterization	Cardiac catheterization that functions as palliation for immature infant until the infant is grown enough for surgery with definitive repair
Invasive ventilation	Delivery of positive pressure ventilation through an invasive interface, this was via an endotracheal tube
Non-invasive ventilation	Delivery of positive pressure ventilation through a noninvasive interface, these were: NIV, NIPPV, CPAP/BIPAP Optiflow or flowsnor were not recognized as ventilation.
Received CPR	Seen as need for chest compressions or need for adrenalin/ cardioversion according to CPR protocol
LCOS, inotropics (including milrinon)	Defined as having the following findings in the first 48 hours postoperatively. - Inotropic (including milrinone) AND

	- At least 2 consecutive lactates > 3 mmol/l AND - Urine output < 1 ml/k/hr during 6 hours
Sepsis	Defined as C-reactive protein (CRP) >20 + positive blood culture OR ≥5 days of antibiotics

B. Tables with additional analyzed variables

Surgical characteristics	No NEC n = 48	NEC n = 14	P value
Type of first intervention			
- Catheterization	19/48 (39.6)	5/13 (38.5)[#]	.941
- Surgery	29/48 (60.4)	8/13 (61.5)[#]	
Surgery delaying cardiac catheterization			
Age at surgery			
- Chronological age(days): median [IQR]	42 [11, 103]	36 [22.5, 64]	.937
- PMA: median [IQR]	37.93 [33.4, 47,1]	37.57 [36.5, 40]	.647
Weight at intervention (grams): median [IQR]	2520 [1945.5, 3885]	2335 [2177, 2850]	.337
Number of hospitalizations: mean ± sd[*]	2.15 (1.03)	2.07 (1.27)	.595

Abbreviations: PMA, post menstrual age

No surgical characteristics were associated with NEC in this study

[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was lower than the true total of patients

[*] Number of hospitalizations had a non-normal distribution, despite this the mean ± sd is reported for illustrative porposes

Preoperative variables	No NEC n =48	NEC n =14	P value
Airway			
Invasive ventilation: n/N (%)	26/48 (54.2)	13/14 (92.9)	**.008**
Duration of 1st invasive ventilation (days): median[IQR]	4 [2, 7]	6 [1.5, 16]	.362
Non-invasive ventilation; n/N (%)	33/48 (68.8)	10/14 (71.4)	.74
Duration of non-invasive ventilation (days): median[IQR]	1.5 [0.5, 12.5]	7.5 [.88, 13.25]	.353
Circulation			
Received CPR: n/N (%)	1/48 (2.1)	3/14 (21.4)	**.010**
CPR duration(days): median [IQR]	2 [2, 2]	10 [10, 10]	.083
Age at CPR*			
- Chronological age (days): median [IQR]	68 [68, 68]	74 (0, -)*	.655
- PMA: median [IQR]	39.85 [39, 39]	43.62[32.3, -]	.655
Arrhythmias: n/N (%)	1/48 (2.1)	1/14 (7.1)	.346
Intubation due to circulatory failure: n/N (%)	14/48 (29.2)	3/14 (21.4)	.568
Inotropics (including milrinon): n/N (%)	16/48 (33.3)	5/14 (35.7)	.615
Pulmonary hypertension: n/N (%)	9/48 (18.8)	4/14 (28.6)	.646
NO treatment: n/N (%)	4/48 (8.3)	0	.452
Renal replacement therapy: n/N (%)	1/48 (2.1)	0	1
Received prostaglandins: n/N (%)	27/48 (56.3)	10/14 (71.4)	.586
Sepsis: n/N (%)	22/48 (45.8)	11/14 (78.6)	**.031**
Neurology			
Seizures: n/N (%)	2/48 (4.2)	0	.438
IVH: n/N (%)	8/48 (16.7)	4/14 (28.6)	.206
IVH grade - I - II - III - IV	 3/8 (37.5) 5/8 (62.5) - -	 2/4 (50) 2/4 (50) - -	.679
PHVD after IVH: n/N (%)	2/8 (25)	0	.368
PVL: n/N (%)	28/48 (58.3)	6/14 (42.9)	.338
PVL grade: n/N (%) - I - II - III - IV	 28 (100) - - -	 6 (100) - - -	-
Cerebellar injury: n/N (%)	0	0	-

Abbreviations: CPR, cardiopulmonary resuscitation; PMA, post menstrual age; NO, nitric oxide; IVH, intra ventricular hemorrhage; PHVD, post hemorrhagic ventricular dilation; PVL, peri ventricular leukomalacia

* 4 infants received CPR of which 3 in the NEC group. The age of 1 infant in the NEC group was unknown. This is illustrated by the hyphen "-"

Postoperative variables	No NEC n = 48	NEC n = 14	P value
Airway			
Invasive ventilation: n/N (%)	48/48 (100)	13/13 (100)[#]	-
Duration of 1st invasive ventilation(days): mean ± sd	3.25 [1, 6]	4 [2, 16.5]	**0.07**
Non-invasive ventilation: n/N (%)	25/47 (53.1)[#]	9/13 (69.2)[#]	.508
Duration of non-invasive ventilation (days): mean ± sd	2 [0.75, 6]	4 [1.75, 28.75]	.148
Circulation			
Received CPR: n/N (%)	3/48 (6.3)	2/13 (15.4)[#]	.287
CPR duration(days): mean ± sd*	2[2,2]	5 [5, 5]	.317
Number of CPR cycles(n): mean ± sd[+]	0	2 [2, 2]	-
Age at CPR			
- Chronological age (days): mean ± sd	80 [0, -]	129 [72, -]	1
- PMA: mean ± sd	38,1 [33, -]	51,2 [41.4, -]	.564
Arrhythmias: n/N (%)	3/48 (6.3)	3/13 (23.1)[#]	.071
LCOS			
- Inotropics 48h postoperatively (incl. milrinon): n/N (%)	22/48 (45.8)	7/13 (53.8)	.701
- 48h postop lactate >3: n/N (%)	6/48 (12.5)	3/13(23.1)	.504
- 48h postop: urine output <1ml/kg/h (6hours): n/N (%)	14/48 (29.2)	4/13 (30.8)	.756
48h postop ECLS: n/N (%)	0	0	-
Signs of PHT on ultrasound: n/N (%)	8/48 (16.7)	6/13 (46.2)	**.038**
iNO treatment: n/N (%)	3/48 (6.3)	5/13 (38.5)	**.002**
Renal replacement therapy: n/N (%)	0	0	-
Sepsis	14/48 (29.2)	6 (46.2)	.247
Neurology			
Seizures: n/N (%)	0	1/13 (7.7)	.053
IVH: n/N (%)	3/48 (6.3)	1/13 (7.7)	.911
IVH grade n/N (%)			
- I	2/3 (66.7)	1 (100)	
- II	-	-	.505
- III	-	-	
- IV	1/3 (33.3)	-	
PHVD: n/N (%)	1/3 (33.3)	0	.505
PVL: n/N (%)	6/48 (12.5)	0	.033
PVL grade n/N (%)			
- I	5/6 (83.3)	0	
- II	1/6 (16.7)	0	-
- III	-	-	
- IV	-	-	
Cerebellar injury: n/N (%)	0	0	-

Abbreviations: CPR, cardiopulmonary resuscitations; PMA, post menstrual age; LCOS, low cardiac output syndrome; PHT, pulmonary hypertension.

[#] n/N (%): number patients meeting variable criteria/ total number of patients with known data. Because of missing data, the N was lower than the true total of patients

* Duration of CPR was only known for 2 infants, one in both the no NEC and NEC group.

[+] Number of CPR cycles was missing for the infants in the no NEC group and known for 1 infant in the NEC group.

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