The pathophysiology of necrotizing enterocolitis in preterm infants

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CHAPTER 2

NECROTIZING ENTEROCOLITIS IN THE NETHERLANDS: AN INCREASED INCIDENCE IN THREE ACADEMIC REFERRAL CENTERS

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Contents

Abstract

Introduction: Necrotizing enterocolitis (NEC) is a severe inflammatory disease with high mortality rates, mostly occurring in preterm infants. The Dutch guidelines for active treatment of extremely preterm infants changed in 2006 from 26+0 to 25+0 weeks of gestation, and in 2010 to 24+0 of gestation. The aim of this study was to gain insight in the incidence, the clinical outcomes and treatment strategies, in three academic referral centers in the Netherlands during the last nine years.

Methods: We performed a multicenter retrospective cohort study of all patients with NEC (Bell stadium ≥ 2a) in three academic referral centers diagnosed between 2005 and 2013. Outcome measures consisted of incidence, changes in clinical presentation, treatment strategies and mortality.

Results: Between 2005 and 2013 14.161 children were admitted to the neonatal intensive care unit in the three centers. The overall percentage of children born at a gestational age of 24 weeks and 25 weeks increased with 1.7% after the introduction of the guidelines in 2006 and 2010. The incidence of NEC increased significantly (period 2005-2007: 2.1%; period 2008-2010 3.9%; period 2011-2013: 3.4%; p=0.001). We observed a significant decrease of peritoneal drainages (↓16%; p=0.001) and a decrease of laparotomies (↓24%; p=0.002). The mortality rate (33% in 2011-2013) remained unchanged.

Conclusion: The incidence of NEC significantly increased in the last nine years. The increase in incidence of NEC seems to be related with the increase of the total admissions of children born at a gestational age of 24- and 25 weeks. The percentage of patients needing surgery decreased, while 30-day mortality did not change.

Introduction

Necrotizing enterocolitis (NEC) is a life-threatening intestinal disease. NEC is characterized by severe intestinal inflammation and necrosis.[1] NEC is mostly seen in very premature infants (≤32 weeks of gestation) and/or in infants with an extreme low birth weight (ELBW <1500 grams). Of the high-risk infants, 7-10% develops NEC.[1–4] In the Netherlands this accounts for around 200 infants each year.[1,5]

NEC is clinically characterized by a distended abdomen, blood per anum, and/or bilious gastric retentions. In a short amount of time intestinal necrosis can develop, followed by intestinal perforation, severe acidosis, shock and death.[1] Subsequently, ±30% of the infants are reported to die due to NEC.[1,6]

The pathophysiology of NEC is still incompletely understood.[7] NEC probably has a multifactorial origin, with prematurity as the most important associated factor. Prematurity goes along with immaturity of the gastrointestinal tract, abnormal bacterial colonization, and an exaggerated inflammatory immune response which all seem to contribute to this disease.[7,8]

Initially, treatment of NEC starts with discontinuing all enteral feedings, nasogastric aspiration, antibiotic therapy and when necessary respiratory and hemodynamic support.[9] Surgical intervention is indicated in case of intestinal perforation or when there is clinical deterioration despite maximal conservative therapy. During surgery the necrotic part of the intestine is resected after which an anastomosis or ostomy is constructed.

In 2006 the Dutch guideline for active treatment of extremely preterm births changed to active treatment from 26+0 to 25+0 weeks of gestation and in 2010 again to 24+0 weeks of gestation.

In the current study we describe the incidence, the clinical presentation, and the outcome of treatment in patients with NEC in three tertiary referral centers. In particularly we focus on NEC in premature infants born at a gestational age of 24- and 25 weeks.[10]

Methods

Patients

This multicenter, retrospective cohort study was performed in the Academic Medical Center Amsterdam (AMC), VU medical Center Amsterdam (VUmc) and the University Medical Center Groningen (UMCG). The diagnosis NEC was made with the use of the modified Bell's staging criteria (Table 1).[11,12] Patients with Bell's stages ≥2A (definite NEC) between January 2005 and December 2013 were included in our study. The medical ethical committee in all three centers approved the use of the clinically acquired data for the purpose of this study and waived written parental informed consent.

Timing of the NEC diagnosis was defined as the moment an abdominal X-ray was obtained when suspicion for NEC arose. The diagnosis NEC was confirmed when pneumatosis intestinalis and/or portal venous gas was present on the abdominal X-ray.

Demographic data

All data were retrospectively collected from medical reports. Demographic data – gestational age, birth weight, sex – were collected. Gestational age and birth weight were classified following the WHO criteria, in which infants born at a gestational age <28 weeks were classified as extreme premature (EP; (11)), and infants born with a birth weight <1500 grams were classified as extreme low birth weight (ELBW; (12)). Thereby, clinical parameters were collected, including severity of NEC (Bell's stage Table 1[11,12]), persistent ductus arteriosus, quantitative hematological/biochemical data (leucocytes, thrombocytes, hemoglobin (Hb), arterial pH and CRP) at the moment of diagnosis, findings on abdominal X-ray during NEC, treatment strategies, and length of treatment.

TABLE 1:

Modified Bell's staging criteria

NEC	Stadium	Symptoms
Suspected NEC	Stadium 1A	Mild abdominal distension Gastric retention Feeding intolerance Heme positive stool Mild ileus on abdominal radiography
Suspected NEC	Stadium 1B	Same as 1A with: Macroscopic blood with stools
Definite NEC	Stadium 2A	Same as 1A with: Increased abdominal distension with gastrointestinal blood loss Pneumatosis intestinalis on abdominal radiography
Definite NEC	Stadium 2B	Same as 2A with: Venous portal gas, possibly with ascites
Advanced NEC	Stadium 3A	Same as 2A with: Characteristics of septic shock Generalized pneumatosis intestinalis with fixated intestinal loops with ascites
Advanced NEC	Stadium 3B	Same as 2A with: Pneumoperitoneum on abdominal radiography

Adapted from Bell MJ, et al (1978), and Walsh MC, et al (1986)

Statistical analysis

The cohort was subdivided in three periods: period 1: 2005-2007, period 2: 2008-2010 and period 3: 2011-2013. The statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 22, IBM Corp., Armonk, New York, USA). For all descriptive data we used mean and standard deviation for homogenous distribution and median with range for heterogeneous distribution. Two-sided p-values <0.05 were considered statistically significant. To test statistical differences between the three time periods, the ANOVA-test with the additional test of Tukey or the Kruskal-Wallis test with the Mann-Whitney U test were used. To test differences between normally distributed variables we used the Student t-test. Differences between categorical variables were analyzed using the Chi-Square test. Risk factors for 30-day mortality were tested with the use of univariate and multivariate analyses, presented with the Odds ratio (OR) and the 95% confidence interval (CI).

Results

Epidemiology

Between 2005 and 2013 a total of 14.161 infants were admitted to the three neonatal intensive care units (NICUs). The percentage of infants born at a gestational age of 24 weeks and 25 weeks increased from 1.7% (n=240) in period 1 to 3.4% (n=481) in period 3 (p=0.01). In total, 441 patients (3.1%) were diagnosed with NEC (Table 2-3). Table 2 presents the epidemiological changes of NEC between 2005 and 2013 in the three NICUs. The overall incidence of NEC increased from 2.1% in period 1 to 3.4% in period 3 (p<0.001). The incidence of NEC in the EP-group (<28 weeks) increased from 6.4% in period 1 to 16% in period 2 and 3 (p=0.001). Further breakdown shows that NEC in the EP-group primarily increased in the group infants born at a gestational age of 24- and 25 weeks: 2.5% (9/359) in period 1 to 5.3% (21/396) in period 2, to 6.8% (30/441) in period 3 (p=0.01).

TABLE 2:

Epidemiology characteristics of NEC from 2005 - 2013

	2005 - 2007	2008 - 2010	2011 - 2013	p-value
NEC, n	99	178	164	0.001[1]
Incidence NEC, % (n)	2.1 (99/4782)	3.9 (178/4518)	3.4 (164/4891)	<0.001[2]
Incidence NEC per center				
Center 1 (n)	2.1 (35/1661)	3.9 (57/1458)	3.5 (57/1632)	0.01[3]
Center 2 (n)	2.5 (42/1697)	3,1(52/1664)	3.7(63/1690)	0.13
Center 3 (n)	1.6 (22/1394)	4.9 (69/1396)	2.8 (44/1569)	0.09
Incidence NEC per gestational age				
<28 weeks, % (n)	6.4 (23/359)	16 (63/396)	16 (71/441)	<0.001[4]
24 & 25 weeks (n)	11 (9/81)	18 (21/118)	18 (30/168)	0.02[5]
28 - 32 weeks % (n)	3.8 (52/1356)	6.3 (81/1288)	5.7 (66/1161)	0.04[6]
>33 weeks, % (n)	0.8 (24/3067)	1.2 (34/2834)	0.8 (27/3289)	0.18
Incidence NEC per birth weight				
< 1000 grams, % (n)	9.2 (40/436)	16 (67/429)	15 (70/469)	0.008[7]
1000 – 1500 grams, % (n)	3.9 (32/826)	7.5 (60/796)	6,8 (50/733)	0.005[8]
1500 – 2500 grams, % (n)	1.3 (19/1430)	2.7 (36/1333)	2.5 (35/1384)	0.03[9]
>2500 grams, % (n)	0.4 (8/2090)	0.8 (15/1960)	0.4 (9/2305)	0.14

1 – Difference between all three time periods: p=0.001
2 – 2005 - 2007 versus 2008 - 2010: p<0.001; 2005 - 2007 versus 2011 - 2013: p<0.001
3 – 2005 - 2007 versus 2008 - 2010: p=0.02; 2008 - 2010 versus 2011 - 2013: p=0.040
4 – 2005 - 2007 versus 2008 - 2010: p=<0.001; 2005 - 2007 versus 2011 - 2013: p<0.001
5 – 2005 - 2007 versus 2008 - 2010: p=0.02; 2005 - 2007 versus 2011 - 2013: p=0.02
6 – 2005 - 2007 versus 2008 - 2010: p=0.04.
7 – 2005 - 2007 versus 2008 - 2010: p=0.01; 2005 - 2007 versus 2011 - 2013: p=0.03
8 – 2005 - 2007 versus 2008 - 2010: p=0.005; 2005 - 2007 versus 2011 - 2013: p=0.04
9 – 2005 - 2007 versus 2008 - 2010: p=0.04

Diagnosis

Between 2005 and 2013 the abdominal X-ray is still the golden standard for the diagnosis of NEC, with pneumatosis intestinalis and/or portal venous gas as characteristics for NEC.

Treatment

Table 3 and 4 show the patient- and treatment characteristics of NEC. In 83% of the patients treatment of NEC was started conservatively with nil per os (NPO), gastric aspiration and broad-spectrum antibiotics directly after the diagnosis. Indications for surgical intervention were intestinal perforation, and clinical deterioration despite maximum conservative therapy (Table 3). In the majority of the cases respiratory and/or hemodynamic support was given (Table 3).

TABLE 3:

Patient characteristics

Clinical variables	2005 - 2007 n=99	2008 - 2010 n=178	2011 - 2013 n=164	p-value
Gestational age (weeks)	29 (24 – 41)	29 (24 – 40)	28 (24 – 39)	0.19
<28 weeks	23 (23)	63 (35)	71 (43)	**0.01**[1]
24 & 25 weeks	9 (8)	21 (12)	30 (18)	**0.001**[2]
28 – 32 weeks	52 (53)	81 (46)	66 (40)	0.20
>33 weeks	24 (24)	34 (19)	27 (17)	0.30
Birth weight (grams)	1160 (540 – 3700)	1200 (430 – 4130)	1070 (410 – 3590)	0.31
< 1000 grams	40 (40)	67 (38)	70 (43)	0.64
1000 – 1500 grams	32 (32)	60 (34)	50 (30)	0.90
1500 – 2500 grams	19 (19)	36 (20)	35 (21)	0.87
>2500 grams	8 (8)	15 (8)	9 (6)	0.06
Male	50 (51)	107 (60)	97 (59)	0.43
Caesarean section	51 (52)	91 (51)	77 (47)	0.75
Persistent ductus arteriosus (PDA)	37 (37)	66 (37)	66 (40)	0.86
Clipping PDA	14 (14)	37(21)	23 (14)	0.68
Laboratory values at NEC diagnosis				
Hb (mmol/L)	8.2 (4.8 – 10.5)	8.0 (4.9 – 14.4)	7.9 (4.8 – 13.3)	0.22
pH	7.3 (6.8 – 7.6)	7.3 (6.7 – 7.6)	7.3 (6.6 – 7.5)	0.05
CRP (mg/L)	27 (1 – 297)	25 (0.6 – 296)	30 (0.3 – 319)	0.57
Leukocytes (10^9/L)	8.6 (0.8 – 46.7)	8.6 (0.6 – 86)	9.2 (1.4 – 59)	0.90
Trombocytes (10^9/L)	193 (4 – 723)	202 (10 – 642)	218 (1 – 780)	0.08
Postconceptional age NEC (weeks)	31 (25 – 42)	31 (25 – 42)	31 (24 – 43)	0.48
Bell's stadium 2	77 (78)	151 (84)	125 (76)	0.15
Bell's stadium 3	22 (22)	27 (16)	39 (24)	
Acute phase mortality (30-day mortality)	33 (33)	43 (24)	41 (25)	0.19
Overall mortality	41 (41)	52 (29)	54 (33)	0.10

** Data are presented as median (range) or in numbers (percentage) unless otherwise stated*

1 – Differences between all three periods; p=0.01

2 – 2005 - 2007 versus 2008 - 2010: p=0.001; 2005 - 2007 versus 2011 - 2013: p=0.001

Surgical treatment

Table 4 shows the details of surgical treatment in NEC. The use of peritoneal drainage decreased significantly (28/99 in period 1, 5/178 in period 2, 20/164 in period 3; p=0.001). The percentage of patients needing a surgical intervention decreased significantly (52/99 cases in period 1, 61/178 cases in period 2, 48/164 cases in period 3; p<0.001). In the EP group there was no significant change in the percentage of patients undergoing surgery (11/23 cases in period 1, 20/63 in period 2, and 25/71 cases in period 3).

TABLE 4:

NEC treatment characteristics

Clinical variables	**2005 - 2007** n=99	**2008 - 2010** n=178	**2011 - 2013** n=164	**p-value**
Inotropes during NEC, n(%)	31 (31)	39 (22)	57 (35)	0.05
Intubation during NEC, n(%)	34 (34)	59 (33)	85 (52)	0.45
Surgical intervention <30 days after diagnosis, n (%)	52 (53)*	61 (34)*	48 (29)*	**<0.001***
Surgical intervention because of pneumopertineum, n(%)#	24 (47)**	34 (56)	30 (63)	**0.006****
Peritoneal drainage, n(%)	28 (28)***	5 (2.8)***	20 (12)***	**<0.001*****
Open/closing procedure, n (%)#	8 (15)	9 (15)	5 (11)	0.76
Primary anastomosis (%)#	18 (35)	25 (41)	23 (47)	0.68
Enterostomy, n(%)#	26 (50)	27 (44)	20 (42)	0.67
Unplanned re-laparotomy (% of total re-laparotomies)	5 (40)	9 (37)	6 (35)	0.57

**2005 - 2007 and 2008 - 2010: p=0.006/ 2005 - 2007 and 2011- 2013: p=<0.001*
***2005 - 2007 and 2008 - 2010: p=0.033/2005 - 2007 and 2011 - 2013: p=0.005*
****2005 - 2007 and 2008 - 2010: p<0.001/2005 - 2007 and 2011 - 2013: p=0.02*
 # % of total surgical interventions
** Data are presented as numbers (percentages). Continuity repairs are included in the planned re-laparotomies.*

Mortality

The acute-phase mortality (30-days) of NEC did not change significantly in the last nine years. Also, the overall mortality did not change over time. In a univariate analysis the following factors seemed to be associated with 30-day mortality: extreme prematurity (<28 weeks; OR 3.9, CI 1.3-12; p=0.02), need for inotropes during NEC (OR 3.1, CI 1.1-8.6; p=0.03), peritoneal drainage (OR 5.9, CI 2.29-15; p=0.001), and surgical interventions during the acute phase of NEC (OR 9.2, CI 1.5-55; p=0.02). In a multivariate analysis only peritoneal drainage was independently associated with 30-day mortality (OR 4.8, CI 1.8-13; p=0.002).

Discussion

In the current study we evaluated the incidence, clinical presentation, and results of treatments of NEC in a 9-year period in three tertiary referral centers in the Netherlands, after modification of the Dutch perinatal guidelines. Since the guidelines changed for active treatment to 24 and 25 weeks of gestation, we focus on these premature infants.

The most important finding of the study is the increased incidence of NEC between 2005 and 2013. This increase in incidence might combine with changes in the guidelines for active treatment in EP-infants. Especially in this group of infants, born at a gestational age of 24 weeks and 25 weeks, a higher incidence of NEC was observed. Another observation was, despite extensive international research on NEC, that the mortality rate of NEC did not change between 2007 and 2013.

During the study period, the incidence of NEC was 3.1% of all NICU-admissions in the three participating academic referral centers. We observed that the incidence of NEC in the EP-group mostly increased in the infants born at a gestational age of 24- and 25 weeks (2.5% in period 1 to 6.8% in period 3). We give two explanations for this increase in incidence. First, in all probability, a reason for the increased incidence of NEC is the increased incidence of infants born at a gestational age of 24 weeks after the change in the 'perinatal management in extreme premature birth' for lowering the active treatment to 24 weeks of gestation. A study on the health outcomes after the change of this guideline a comparable incidence of NEC was reported for infants born at 24- and 25 weeks of gestation.[14] A second explanation for the increase in incidence of NEC is the improved early survival of infants who otherwise would been deceased before the reach of the typical postmenstrual age when NEC normally presents.[15]

NEC effects 10% of the EP-infants, of which 1 out of 3 deceases.[16] In accordance with the literature[2] we report a 30-day mortality of respectively 33%, 24% and 25% over the three periods during 2005-2013. Despite the high morbidity and mortality, currently, adequate preventive- and treatment strategies are not yet available for

clinical practice. After 2005 there were no major changes in the conservative treatment of NEC in the three tertiary referral centers. The addition of probiotics to nutrition is an upcoming experimental preventive therapy. However, there are still questions that need to be answered regarding the use and safety of probiotics before the use of probiotics can be implemented in standard clinical treatment. Currently, probiotics are not used in clinical practice in one of the three participating centers.
We observed that the percentage of surgical interventions decreased between 2005 and 2013 (a decrease of 53% to 29%). This percentage is similar to reports from literature in which a percentage between 20 and 40% surgical interventions in the acute phase of NEC is described.[16] While it is plausible that this trend is primarily due to the increase of the EP-group of total NICU admissions – the group of infants in which health practitioners are generally restrained with surgical interventions – we did not see a decrease of infants who underwent surgery due to NEC in the EP-group after 2007.

In the past nine years, the use of peritoneal drainage in critically ill infants decreased. The use of peritoneal drainage is controversial, and is mostly used as a temporary measure to stabilize the infant before surgery.[2,16–18] Our study shows that the use of peritoneal drainage has significantly decreased (from 28% to 12%). Thereby, we observed that the use of peritoneal drainage is associated with 30-day mortality. In all probability the association between peritoneal drainage and mortality is most likely a reflection of the use of peritoneal drainage in the most severely ill children in an attempt to stabilize infants who are too instable for surgical intervention.

Preventive measures to prevent the development of NEC mainly focus on maternal donor milk and the supplementation of pre- and probiotics to the neonatal feeding of the vulnerable infant. A diet with exclusively mother's milk for premature infants until the postmenstrual age of 33 weeks has a protective effect against NEC.[17] Thereby, the use of pre- and probiotics might have positive effects protecting the infant from NEC development.[17,18] A recent Cochrane review of Alfaleh et al.[18] concluded that the supplementation of probiotics decreased the incidence of NEC in infants with a birth weight >1500 grams. However, the benefits and the possible side-effects of probiotics in infants born with a birth weight <1000 grams, the infants with the highest risk on NEC development, are still not fully elucidated.[18] More studies are needed to elucidate the safety and effectiveness, and especially to reveal the exact composition of the probiotics.[9]

The current study has its limitations. First, the retrospective nature of the study could have led to historical data loss. A second limitation is the composition of the cohort. We only included NEC patients in our cohort, without the use of a (healthy) control group.

Conclusion

This retrospective cohort study shows an increased incidence of NEC after, among others, since the guidelines changed for active treatment to 24 and 25 weeks of gestation. Thereby, this study shows that the total of surgical interventions decreased, but the mortality did not change during the last nine years. Further research should be done to develop new and/or improve preventive- and treatment strategies against NEC.

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SECTION 2

INTESTINAL BARRIER INTEGRITY AND CIRCULATION

Chapter 3 Paneth cells in the developing gut: when do they arise and when are they immune competent?

Chapter 4 Tissue oxygenation and intestinal fatty acid-binding protein in plasma during necrotizing enterocolitis

Chapter 5 Intestinal fatty acid-binding protein levels in necrotizing enterocolitis correlate with extent of necrotic bowel: results from a multicenter study