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Necrotising enterocolitis: is there a relationship to specific pathogens?

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Abstract Outbreaks of necrotising enterocolitis (NEC) have often been related to specific pathogens such as *Enterobacteriaceae*. This relationship, however, remains uncertain because of the retrospective nature of the studies addressing this issue. We performed a prospective study to investigate whether there is indeed an association between NEC and specific pathogens. Between April 1993 and March 1997, stools of neonates of < 36 weeks admitted to our neonatal unit were investigated for bacteria in weekly intervals. Clinical and bacteriological data from each infant who developed NEC were compared with those from two control infants matched for gestational age and date of admission. Eighteen infants developed 19 episodes of NEC (clinical signs + air in portal vein); 8 of these had laparotomy; two died. Occurences of NEC were homogeneously distributed over the 4-year study period. The only significant differences in the clinical course prior to NEC were a more severe stage of respiratory distress syndrome [median 2 (0–4) vs. 0 (0–3), $P < 0.05$] and a higher proportion of infants who had only been formula fed (63 vs. 32%, $P < 0.05$) in the cases. Within the last week prior to NEC, potentially pathogenic bacteria were identified in stools of all cases and 79% of controls ($P < 0.05$). However, there was no significant difference in the occurrence of specific pathogens or groups of pathogens in cases compared with controls.

Conclusion Although gut colonisation with potential pathogens appeared to be a prerequisite for the development of NEC, there were no specific bacteria associated with this disease if data from infants with NEC were compared with those from time- and gestational age-matched controls.

Key words Necrotising enterocolitis · Pathogens · Temporal distribution

Abbreviations *NEC* necrotising enterocolitis · *NICU* neonatal intensive care unit

Introduction

Necrotising enterocolitis (NEC) has an incidence of 2%–5% in all preterm infants and up to 13% in those weighing < 1500 g at birth [5, 14, 25]. It accounts for a considerable proportion of deaths in neonatal intensive care units (NICU). Reported mortality rates vary between 10% and 55% [11, 20]. The aetiology of NEC remains unknown. Observations of an apparent clus-

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Table 1 Clinical histories of the infants with necrotising enterocolitis (NEC) (the one infant with two NEC episodes is counted twice) and their matched controls. Continuous variables are reported as medians (and ranges)

	Cases (n = 19)	Controls (n = 38)
Gestational age (weeks)	30 (25–34)	30 (25–35)
Birth weight (g)	1070 (460–1900)	1300 (555–2650)
Apgar score 5 min	8 (5–10)	8 (2–10)
Arterial cord blood pH	7.26 (7.06–7.34)	7.31 (6.83–7.46)
Antenatal steroids	14 (74%)[a]	31 (84%)
Patent ductus arteriosus	1 (5%)	2 (5%)
Duration of IPPV (days)	1 (0–32)	0 (0–21)
Maximum grade of RDS	2 (0–4)	0 (0–3)*
Last haemoglobin prior to NEC (g/dl)	13.4 (9.6–18.4)	13.0 (8.0–18.7)
Onset of feeding (days)	2 (1–20)	2 (1–6)
Type of milk given:		
Breast milk only	3 (16%)	4 (12%)
Breast milk and formula	4 (21%)	21 (56%)
Formula only	12 (63%)	12 (32%)*
Average daily increment (ml/kg per day)	7.2 (0–15.4)	6.1 (0–21)
Antibiotics given prior to NEC (days)	8 (0–57)	6 (0–25)

* $P < 0.05$
[a] Complete course of betamethasone in 12 infants

tering of cases led to the hypothesis that NEC is causally related to specific infectious agents, and this was seemingly confirmed by several uncontrolled case series identifying pathogens such as *Enterobacteriaceae* [3, 9, 19, 21], *Clostridia spp*. [15], coagulase-negative staphylococci [23], rotavirus [22] or single species retrieval during disease outbreaks [6]. The problem with these studies, however, is that there are time dependent variations in the colonisation of infants treated in a NICU, making it difficult to determine whether the identification of a specific pathogen in a retrospective case series is indicative of a causal relationship between this pathogen and NEC, or whether it merely reflects the general pattern of pathogen distribution in the NICU at the time of study. Moreover, the apparent clustering of NEC cases was not subjected to formal statistical tests.

We conducted a prospective case-control study to answer the following questions: (i) is there an association between NEC and specific pathogens, and (ii) are there specific clinical risk factors that would help to identify infants at risk of developing NEC.

Patients and methods

Between April 1, 1993 and March 31, 1997, stool samples were collected at weekly intervals from all infants born at <36 weeks gestation and admitted to the NICU at Hannover Medical School immediately after birth. The study group consisted of all infants who developed definite NEC during the study period, defined as the presence of one or more clinical signs (abdominal distension, feeding intolerance, blood in stool) plus the ultrasound and/or radiological demonstration of gas in the liver and/or portal vein (i.e., Bell stage 2) [4]. For each of these cases two control infants without evidence of NEC were selected, matched for gestational age at birth and date of admission, with matching priority given to gestational age in one infant and to date of admission in the other. Control infants had to be present in the NICU at the time of diagnosis of NEC in the matching case subject.

Stool specimens were plated on Columbia blood, MacConkey and Preston agar and several *Salmonella* selective media and incubated at 37°C. In addition, a tetrathionate broth and a sorbitol MacConkey agar were inoculated and incubated at 37°C. All media were screened for bacterial growth for at least 48 h. More than two morphologically different *Enterobacteriaceae* were summarised as mixed Gram negative culture. Gram positive bacteria were identified to genus level, pathogenic bacteria like *Staphylococcus aureus* and beta-haemolytic streptococci to the species level according to standard laboratory protocols. Non-pathogenic bacteria like *S. bifidum* were disregarded. In cases, the last stool culture prior to diagnosis of NEC was taken for analysis; in controls, the last stool culture obtained prior to reaching the respective postnatal age of the case infant at the time of onset of NEC was taken for analysis. Viruses were looked for using electron microscopy. For the laboratory data, the extreme values measured during the first 72 h following diagnosis of NEC (or the respective control period) were taken.

Statistical analysis was performed using SPSS 6.0 for Windows. Chi-square and Wilcoxon matched pairs tests were computed. Uniformity of the temporal distribution of NEC occurrences was tested using the Kolmogorov-Smirnov test. A P-value of <0.05 was considered statistically significant.

Results

Of 436 infants <36 weeks admitted to the NICU throughout the 4-year study period, 18 (4%) developed 19 episodes of NEC. Thirteen of 19 episodes occurred in five periods of 1–4 weeks duration each. Uniformity testing, however, did not confirm the clinical impression that NEC cases occurred in clusters ($P = 0.28$). Age at presentation varied between 4 and 74 days (median 19 days) and was inversely correlated with gestational age at birth ($r = -0.65$, $P < 0.01$). Clinical signs included feeding intolerance with and without gastric residuals (8/19), abdominal distension (16/19), and blood in stools (15/19). Predominant laboratory findings were increased C-reactive protein (>5 mg/l, 19/19), leucopenia (<5.000/µl) or leucocytosis (>20.000/µl, 12/19), thrombocytopenia (<150.000/µl, 8/19) and hyponatraemia (<131 mmol/l, 8/19). Ultrasound investigations revealed gas bubbles in the liver and/or portal vein

Table 2 Individual organisms identified in stool cultures from infants with NEC and their matched controls

	Cases (n = 19)	Controls (n = 38)
Clostridium diff.	0	1
Corynebac. spp.	0	1
Enterococcus faec.	4	4
Enterococcus spp.	6	13
Staph. aureus	2	3
Staph. coag. neg.	6	10
Non-haemolytic Strept.	0	1
Strept. viridans	0	2
Actinobacter baumanii	0	3
Citrobacter freundii	0	1
E. coli	2	2
Enterobacter aggl.	0	1
Enterobacter cloacae	5	4
Kleb. oxytoca	2	1
Kleb. pneumoniae	3	7
Neisseria spp.	0	1
Other Gram neg. organisms	1	3
Aerobe spore-forming organism	0	1
Candida albicans	1	3
Yeasts	1	1

in all 19 episodes (no infant had a catheter in the vena cava), while radiological demonstration of pneumatosis intestinalis was present in only 13 of these episodes. Laparotomy was performed in eight cases of NEC with intestinal perforation being found in two. Histology invariably showed results compatible with a diagnosis of NEC. Two infants (none of those with perforation) died of fulminant NEC with multiorgan failure due to severe septicaemia.

Comparison of the clinical histories prior to NEC showed no significant differences between cases and controls except for a significantly more severe degree of respiratory distress and a higher proportion of infants who had been exclusively formula fed in the cases (Table 1). No infant had received oral immunoglobulins [10], and none had congenital heart disease. Only three infants with NEC were receiving antibiotics at the time of diagnosis [ampicillin/tobramycin (1), cefotaxim/vancomycin (2)].

Bacteriological data from the last 7 days prior to NEC were available in all cases and controls; in 15 infants with NEC, these were from stools sampled within ≤48 h prior to diagnosis. All stools from cases grew potential pathogens, whereas 8/38 controls (21%) did not ($P < 0.05$). The distribution of bacteria found in stools from both cases and controls is shown in Tables 2 and 3. Electron microscopy was positive for rotavirus in one infant with NEC and in no control infant. Statistical analysis showed no significant differences for any specific pathogen or group of pathogens between cases and controls (Table 3).

Table 3 Results of stool cultures from infants with NEC and their matched controls, grouped by species. Prevalence data are given with 95% confidence intervals

	Cases (n = 19)	Controls (n = 38)
No potential pathogen	0 (0%; 0–0)	8 (21%; 8–34)*
Gram positive spp.	14 (74%; 54–94)	26 (68%; 53–83)
Enterobacteriaceae	10 (53%; 31–75)	12 (32%; 17–47)
Glucose non-fermenting Gram neg. rods	0 (0%; 0–0)	3 (8%; −1–17)
Candida spp.	2 (11%; −3–25)	4 (11%; 1–21)

* $P < 0.05$

Discussion

The main results of this study can be summarised as follows: (i) age at occurrence of NEC was inversely correlated with gestational age at birth, (ii) there was no significant characteristic feature in the clinical course prior to NEC except for a more severe respiratory distress syndrome and a higher proportion of infants who were exclusively formula fed in the cases, and (iii) there was no specific pathogen or group of pathogens when stools from infants with NEC, collected within 1 week prior to disease outbreak, were compared with those from time- and gestational age-matched controls. This was despite the fact that gut colonisation with potential pathogens appeared to be a prerequisite for the development of NEC.

The incidence of NEC observed in this study was within the range found by other investigators [5, 14]. Considering that our study included only infants with gas in the portal venous system, which is highly specific for NEC [18, 24] and is widely regarded as a sign of severe disease, both our mortality rate and our rate of intestinal perforation (11% each) are quite low [1, 14, 25]. This may be related to the frequent use of ultrasound on our unit which is more sensitive to the detection of gas bubbles in the portal venous system than abdominal X-ray films [18, 24] and may thus have allowed for an earlier diagnosis and thereby therapy of NEC. The latter statement, however, remains somewhat speculative as long as the exact sensitivity and specificity of the various radiological and sonographic signs of NEC have not been formally studied.

A large number of risk factors for NEC have been identified, including perinatal asphyxia [2], patent ductus arteriosus [12], early and/or rapid feeding advancements [17], formula feeding [2, 16], and respiratory distress syndrome [2]. Only the latter two factors were confirmed in this study, which may be due to our limited sample size or to the fact that we matched for gestational age at birth, thereby eliminating risk factors that are merely related to immaturity rather than specifically to NEC, as also suggested by other investigators [26]. Umbilical artery catheters, another potential risk factor for NEC [8], could not be addressed in this study as arterial access on our unit is usually achieved via peripheral catheters.

A later occurrence of NEC in less mature infants has already been reported [2, 24], suggesting that there is a gestational- rather than postnatal-age dependent phase of increased gut vulnerability during which NEC is more likely to occur.

We did not observe the "typical" clustering of NEC reported by other investigators [7, 8, 21]. None of these earlier studies, however, applied statistical methods to prove that NEC occurrences were indeed non-homogenously distributed over time (the latter would suggest that NEC is an infectious disease). We did also not identify any specific pathogen or group of pathogens to occur more often in cases than in time- and age-matched controls, confirming results from an earlier, albeit retrospective, study [13]. The pathogens identified in other retrospective case series during apparent disease outbreaks [16, 19, 21, 23] varied considerably, suggesting that it is not one specific pathogen that is responsible for the disease, but that various pathogens can cause NEC in a sufficiently vulnerable host. Taken together, these results support the hypothesis that NEC is caused by a combination of feeding and ischaemia, with pathogens only playing a secondary role in the disease process.

Our study does not support the hypothesis that specific pathogens cause NEC. We cannot, however, exlude any such association, since this study only had a power of 80% to detect an association between NEC and specific pathogens with a relative risk of 4.6 or greater (P_0 0.5, α 0.05 1-sided, β 0.2). Had we aimed to identify a risk factor with a lower effect estimate, e.g. around 2, a sample size of 107 cases and 107 controls would have been required. This was considered impossible in a prospective single-centre study. It was also not considered necessary, since a risk factor with such a low strength, if it exists, is unlikely to result in any clinical consequences. We were also unable to determine whether there were any changes in gut colonisation immediately (≤2 days) prior to NEC, as suggested by a recent study from England [14].

In conclusion, by prospectively collecting data on gut colonisation in a level III NICU over a 4-year period, we could not demonstrate a clustering of NEC cases or any significant difference in gut colonisation between infants who developed NEC and time- and age-matched controls. These data do not support the concept that NEC is related to a specific pathogen or group of pathogens.

References

1. Alvarez EM, Corral GM, Oliveira F, Kurlat I (1997) Necrotizing enterocolitis (NEC): can perforation be predicted? Pediatr Res 41:135A
2. Beeby PJ, Jeffery H (1992) Risk factors for necrotising enterocolitis: the influence of gestational age. Arch Dis Child 67:432–435
3. Bell MJ, Feigin RD, Ternberg JL, Brotherton T (1978) Evaluation of gastrointestinal microflora in necrotizing enterocolitis. J Pediatr 92:589–592
4. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T (1978) Neonatal necrotizing enterocolotis. Ann Surg 187:1–7
5. Bell MJ, Shackelford P, Feigin RD, Ternberg JL, Brotherton T (1979) Epidemiologic and bacteriologic evaluation of neonatal necrotizing enterocolitis. J Pediatr Surg 14:1–4
6. Bell MJ, Rudinsky M, Brotherton T, Schroeder K, Boxerman SB (1984) Gastrointestinal microecology in the critically ill neonate. J Pediatr Surg 19:745–751
7. Book LS, Overall JC, Herbst JJ, Britt MR, Epstein B, Jung AL (1977) Clustering of necrotizing enterocolitis. N Engl J Med 297:984–986
8. British Association for Perinatal Paediatrics (1983) Surveillance of necrotising enterocolitis, 1981-2. BMJ 287:824–826
9. Cushing AH (1983) Necrotising enterocolitis with eschericia coli heat-labile enterotoxin. Pediatrics 71:626–630
10. Eibl MM, Wolf HM, Fürnkranz H, Rosenkranz A (1988) Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA-IgG feeding. N Engl J Med 319:1–7
11. Gortner L, Limmer J, Pohlandt F, Bartmann P, Kelsch G (1995) Die nekrotisierende Enterokolitis: eine 12-Jahres-Retrospektive. Klin Pädiatr 207:28–33
12. Grosfeld JL, Chaet M, Molinari F, Engle W, Engum SA, West KW, Rescorla FJ, Scherer LR (1996) Increased risk of necrotizing enterocolitis in premature infants with patent ductus arteriosus treated with indomethacin. Ann Surg 224:350–357
13. Gupta S, Panigrahi P, Natero J, Patel B, Guers L, Morris JG, Gewolb IH (1992) Stool bacterial plasmid profiles in infants with necrotising enterocolitis. Pediatr Res 31:204A
14. Hoy C, Millar MR, MacKay P, Godwin PGR, Langdale V, Levene MI (1990) Quantitative changes in faecal microflora preceding necrotising enterocolitis in premature neonates. Arch Dis Child 65:1057–1059
15. Kosloske AM, Ball WS, Umland E, Skipper B (1985) Clostridial necrotizing enterocolitis. J Pediatr Surg 20:155–159
16. Lucas A, Cole TJ (1990) Breast milk and neonatal necrotising enterocolitis. Lancet 336:1519–1523
17. McKeown RE, Marsh TD, Amarnath U (1992) Role of delayed feeding and of feeding increments in necrotizing enterocolitis. J Pediatr 121:764–770
18. Merritt CRB, Goldsmith JP, Sharp MJ (1984) Sonographic detection of portal venous gas in infants with necrotizing enterocolitis. Am J Roentgenol 143:1059–1062
19. Millar MR, MacKay P, Levene M, Langdale V, Martin C (1992) Enterobacteriaceae and neonatal necrotising enterocolitis. Arch Dis Child 67:53–56
20. Panigrahi P, Gupta S, Gewolb IH, Morris JG (1994) Occurrence of necrotizing enterocolitis may be dependent on patterns of bacterial adherence and intestinal colonization: studies in Caco-2 tissue culture and weanling rabbit models. Pediatr Res 36:115–121
21. Powell J, Bureau MA, Pare C, Gaildry M-L, Cabana D, Patriquin H (1980) Necrotizing enterocolitis. Am J Dis Child 134:1152–1154
22. Rotbart HA, Nelson WL, Glode MP, Triffon TC, Kogut SJH, Yolken RH, Hernandez JA, Levin MJ (1988) Neonatal rotavirus-associated necrotising enterocolitis: case control study and prospective surveillance during an outbreak. J Pediatr 112:87–93
23. Scheifele DW (1990) Role of bacterial toxins in neonatal necrotising enterocolitis. J Pediatr 117: S44–S46
24. Schmidt H, Schramm A (1994) Aussagefähigkeit und Stellenwert von bildgebender Diagnostik und Duplexsonographie bei Neugeborenen mit nekrotisierender Enterokolitis. Fortschr Röntgenstr 160:524–530
25. Stanley MD, Null DM (1977) Relationship between intestinal colonization with specific bacteria and the development of necrotizing enterocolitis. Pediatr Res 11:543
26. Yu VYH, Joseph R, Bajuk B, Orgill A, Astbury J (1984) Perinatal risk factors for necrotizing enterocolitis. Arch Dis Child 59:430–434