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AN OUTBREAK OF NECROTIZING ENTEROCOLITIS

ASSOCIATION WITH TRANSFUSIONS OF PACKED RED BLOOD CELLS

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Of 187 newborns admitted to a 33-bed, level III neonatal intensive care unit between January 1, 1985 and June 23, 1985, 33 developed necrotizing enterocolitis during their hospital stay. Twenty of the 33 newborns (61%) had onset of symptoms between April 1 and June 23, suggesting clustering during this period. A case-control study, with matching on birth weight class, approximate date of admission to the unit and approximate duration of stay, failed to reveal any association of the syndrome with type or timing of feeding, perinatal hypoxic events, as determined by apgar scores and labor history, or specific microbial organisms. By contrast, however, transfusion of packed red blood cells was highly and significantly associated with the syndrome (odds ratio = 15.1, 95% confidence interval = 2.59–92.51). In addition, therapy with caffeine, with theophylline, and with furosemide were moderately associated with the syndrome, although not significantly so. During this outbreak period, the incidence of necrotizing enterocolitis by birth weight was 30.6% in infants less than 1,500 gm, 10.8% in infants 1,500–2,500 gm, and 11.9% in infants 2,500 gm or more. These findings confirm the importance of low birth weight as a risk factor for development of the syndrome and suggest that insults to volume homeostasis, such as transfusion and use of diuretics, need to be considered as possible mechanisms whereby necrotizing enterocolitis is initiated.

blood transfusion; disease outbreaks; diuretics; enterocolitis, necrotizing; infant, premature; space-time clustering

Neonatal necrotizing enterocolitis is relatively common among premature infants. It has been estimated that the syndrome occurs in 1–8 per cent of all infants admitted to neonatal intensive care units, and that the overall incidence is approximately 1 per 1,000 live births (1–3). Pathologically, necrotizing enterocolitis is characterized by necrosis of gastrointestinal mucosa (4), and, clinically, by systemic and gastrointestinal signs and symptoms including blood in the stool (gross or microscopic), abdom-

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inal distention, and roentgenographic evidence of either intraperitoneal air, gas in the intestinal wall (pneumatosis intestinalis), or hepatic portal gas (5, 6). The etiology and pathogenesis of this syndrome are not well established; however, several hypotheses have been formulated (7–11). The major hypotheses emphasize either 1) the role of gastrointestinal tract ischemia resulting from predisposing factors such as birth asphyxia, 2) infection, or 3) immaturity of gastrointestinal functioning.

A cluster of necrotizing enterocolitis cases in a 33-bed, level III neonatal intensive care unit in Oklahoma City, Oklahoma was recognized in May 1985. From January 1 through May 30, 1985, 27 clinically suspect cases of the syndrome were diagnosed; three cases had been diagnosed over the same period in the previous year. An epidemiologic investigation of this cluster of cases was undertaken in June 1985.

Materials and methods

Case definition and case finding

An infant was classified as a confirmed case of necrotizing enterocolitis if that infant was admitted to the neonatal intensive care unit between January 1, 1985 and June 23, 1985, was systemically ill, and had roentgenographic evidence of the syndrome (pneumatosis intestinalis, hepatic portal gas, or free intraperitoneal air). An infant admitted during that time period who had gross or occult blood detected in his/her stool (three stools on one day or one stool on two consecutive days), who was systemically ill, and who had one or more of the following signs was classified as a suspected case: 1) abdominal tenderness, 2) abdominal distention, 3) vomiting, 4) gastric residual, 5) hypoglycemia or hyperglycemia, 6) apnea, 7) temperature instability, and 8) lethargy.

The daily log of admissions to the neonatal intensive care unit, kept by infection control personnel, was used to identify all possible cases that occurred within the defined time period. Hospital charts of possible cases were reviewed and infants were classified, according to the criteria noted, as confirmed cases, suspected cases or not necrotizing enterocolitis.

Selection of controls

Control infants were identified from the same neonatal intensive care unit admissions log kept by infection control personnel. The log provided date of admission, birth weight, and date of discharge. The controls selected were matched to cases on three criteria: 1) approximate birth weight as given by birth weight class, 2) approximate duration of stay in the unit, and 3) approximate date of admission to the unit. The birth weight classes were: less than 1,000 gm, 1,000–1,499 gm, 1,500–1,999 gm, 2,000–2,499 gm, 2,500–2,999 gm, 3,000–3,499 gm, and 3,500 gm or heavier.

A control was assigned as a match to a case if the control was in the same birth weight class as the case, if the control's duration of stay in the unit was at least 60 per cent as long as the time from birth to onset of the syndrome in the case, and if the control was admitted within 30 days of the case's date of admission. At least two controls per case were sought.

Data collection

A questionnaire for demographic information, information on exposures previously reported as risk factors for necrotizing enterocolitis, and information on other exposures was prepared before the hospital charts of case and control infants were reviewed. All exposures were determined only for the interval from birth to onset of the syndrome in the cases, and for the same duration of time from birth in the corresponding controls.

Microbiologic methods

Cases which occurred before June were not systematically investigated. In general, stool cultures, blood cultures, and peritoneal fluid cultures (in the case of intestinal perforation followed by laparotomy) were obtained and processed following routine methods in the hospital laboratory. The

cluster of cases in June was more extensively investigated for potential gastrointestinal pathogens. Stools were obtained from four confirmed case infants, from nine suspected case infants, and from 17 asymptomatic infants exposed to a case between June 9 and June 15 by residence in the same nursery (these cases had onset of symptoms between June 6 and June 12). These specimens were cultured aerobically for *Salmonella*, *Shigella*, and *Yersinia*, for *Campylobacter* (using campy blood agar), for *Clostridium difficile* using cycloserine-cefoxitin-fructose agar (CCFA), and for *Aeromonas* species using blood agar containing ampicillin (10 μg/ml). All stool samples were examined for *Cryptosporidium* by the sugar-flotation method. Specimens from two confirmed case infants, five suspected case infants, and five asymptomatic exposed infants were examined by electron microscopy for viruses. Viral cultures of stools were not performed; several specimens were examined for rotavirus by immunoassay (Rotazyme®, Abbott Laboratories, N. Chicago, IL), using a confirmatory blocking step.

Analytic methods

The frequency distributions of descriptive variables for cases were compared with those for controls using the chi-square statistic. Necrotizing enterocolitis-exposure associations in the data were initially examined by the Mantel-Haenszel method of matched analysis with variable numbers of controls per case (12). A stratified analysis of the unmatched data was performed before fitting a matched logistic regression model (13, 14). Power calculations were performed according to Schlesselman (12) using information from all one-to-one matches and from other matches where all controls were concordant with respect to the exposure being examined.

RESULTS

Characteristics of study subjects

From January 1, 1985 through June 23, 1985, necrotizing enterocolitis was diagnosed in 33 of 187 newborns admitted to the neonatal intensive care unit. Twelve of these 33 newborns were classified as confirmed cases and the remaining 21 as suspected cases. Suspected cases did not differ significantly from confirmed cases with respect to birth weight, sex, or gestational age. Median birth weights were 1,360 gm and 1,250 gm, respectively, for confirmed and suspected cases. The median gestational age of infants classified as confirmed cases was 32.5 weeks (range, 26–40 weeks), and the median gestational age of infants classified as suspected cases was 28.5 weeks (range, 26–38 weeks). Nine of 12 infants classified as confirmed cases and 12 of 21 infants classified as suspected cases were male.

The frequencies of signs and symptoms of necrotizing enterocolitis were similar in confirmed and suspected cases (chi-square = 8.148, df = 10, $p = 0.384$). Surgical management of the syndrome was necessary in five infants classified as confirmed cases. One death occurred in the confirmed case group and two deaths in the suspected case group. Because of the overall similarity of the two groups, no further distinction will be made between them.

Forty controls were identified. For 16 cases, only one matching control was found; two or three controls were identified for 11 cases; no controls could be found for six of the cases. Cases and controls were not significantly different in regard to race, sex, or gestational age (table 1).

Clustering and control measures

A definite cluster of cases occurred in May and June, with a suggestion of clustering in April (figure 1). During the first five months of 1984, the incidence of necrotizing enterocolitis was 0.31 cases per 1,000 patient-days. By contrast, during the first five months of 1985, the incidence was 2.12 cases per 1,000 patient-days.

Considering the birth weight-specific incidence for the period of January 1 to June 23 (table 2), the hypothesis that birth weight and development of the syndrome

TABLE 1

Characteristics of cases and controls outbreak of necrotizing enterocolitis, Oklahoma City, Oklahoma, January–June 1985

Characteristic	Cases		Controls	
	No	(%)	No	(%)
Race				
White	22	(66.7%)	27	(73.0%)
Black	8	(24 2%)	6	(16.2%)
Mexican	1	(3.0%)	2	(5.4%)
Indian	2	(6.1%)	2	(5.4%)
Total	33		37	
	$\chi^2 = 0.9036$, df = 3			
Sex				
Male	21	(63.6%)	28	(70 0%)
Female	12	(36.4%)	12	(30.0%)
Total	33		40	
	$\chi^2 = 0.3318$, df = 1			
Gestational age (weeks)				
<28	6	(19.4%)	8	(20.5%)
28–32	13	(41.9%)	11	(28.2%)
33–36	5	(16 1%)	9	(23.1%)
≥37	7	(22.6%)	11	(28.2%)
Total	31		39	
	$\chi^2 = 1.5906$, df = 3			

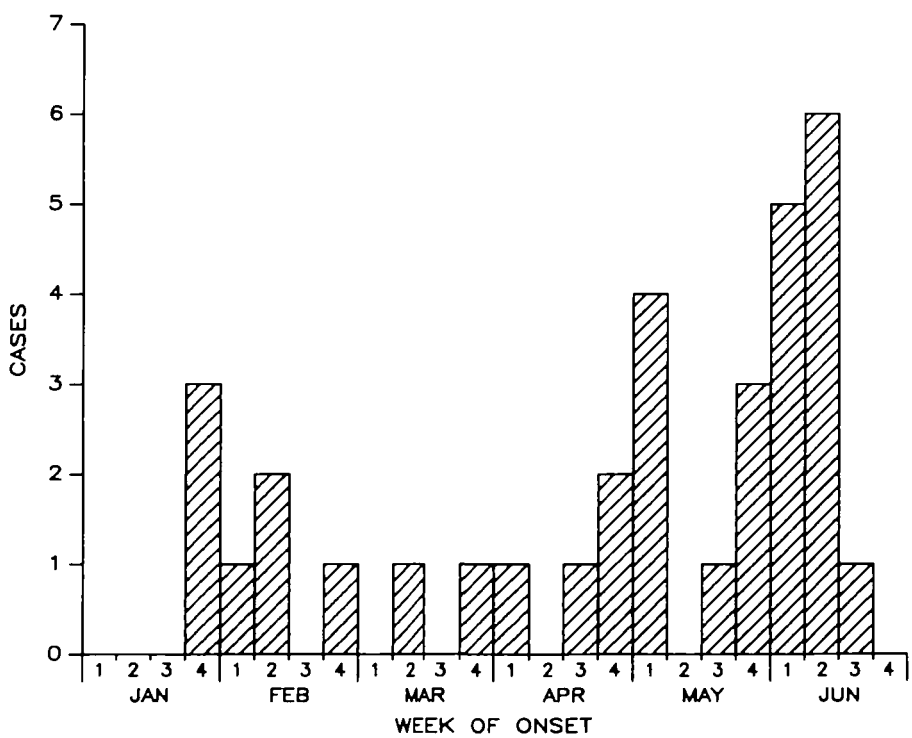

FIGURE 1 Cases of necrotizing enterocolitis by week of onset, January–June 1985, Oklahoma City, Oklahoma.

TABLE 2

*Birth weight-specific incidence of necrotizing enterocolitis, Oklahoma City, Oklahoma, January 1, 1985–June 23, 1985**

Birth weight category (gm)	No. of cases	No. of admissions	Incidence (per 100)
<1,000	4	27	14.8
1,000–1,499	15	35	42.9
1,500–1,999	3	29	10.3
2,000–2,499	4	36	11 1
2,500–2,999	3	22	13.6
3,000–3,499	2	20	10.0
>3,499	2	17	11 8

* Note: one of the 187 admissions had no recorded birth weight.

are independent can be rejected ($\chi^2 = 18.95$, df = 6, $p = 0.0046$). The increased risk of the disease among infants with birth weights of 1,000–1,499 gm is striking.

Before June, personnel who cared for infants with necrotizing enterocolitis were required to wear gloves and overgowns when handling affected infants. With the continued development of new cases, these "enteric precautions" were continued, and on June 9, 1985 a cohort system was initiated in the unit. Separate cohorts of 1) symptomatic infants, 2) infants exposed to symptomatic infants, and 3) nonexposed infants were established in separate rooms in the unit. In addition, no admissions to the unit were allowed until June 26. At that time, a room for newly admitted infants was established and the isolation of exposed, asymptomatic infants from nonexposed infants was discontinued. An attempt was made to establish a cohort of nurses for each cohort of infants for every shift. However, some nurses, all house staff, and other personnel (e.g., respiratory therapists and phlebotomists) had to break the cohorting scheme.

Microbiologic results

No ill or exposed infants had *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, or *Aeromonas* species isolated from feces. Two of 13 cases had *Clostridium difficile* isolated from stools, compared with four of 17 exposed infants. *Staphylococcus epidermidis* was found in the feces of 11 of 13 cases and eight of 17 exposed infants ($\chi^2 = 3.64$, $p = 0.059$). This finding may reflect the fact that all cases tested had received antibiotics (ampicillin and gentamicin) for at least two days before collection of the stool specimen. One case and one exposed infant had coronavirus-like particles detected on electron microscopy of feces; no other viral particles were identified.

Risk factor analysis

Of the exposures examined in this investigation as possible risk factors for development of necrotizing enterocolitis, four were moderately or strongly associated with the syndrome: transfusion of packed red blood cells, and therapy with furosemide, caffeine, or theophylline. Odds ratios for these exposures were 15.5, 5.5, 5.7, and 4.5, respectively (table 3). The association was statistically significant only for transfusion; however, therapy with caffeine approached significance. Of 29 cases for whom transfusion status was determined, 24 (82.8 per cent) had one or more transfusions of packed red blood cells before onset of necrotizing enterocolitis. Six of 31 cases (19.4 per cent) were treated with caffeine, four of 30 (13.3 per cent) with theophylline, and seven of 31 (22.6 per cent) with furosemide.

During the outbreak period, a nearby level II nursery, serving a large obstetric facility, accounted for 40 per cent of all neonatal intensive care unit admissions. Twenty-five of 33 case infants and 22 of 40 control infants were referred from the nearby nursery. The variable, hospital of birth, distinguished infants born at the nearby nursery from those born at all other hospitals which referred newborns to the neonatal intensive care unit during this time. The odds ratio for hospital of birth was 1.90 (table 3).

The model fitted by matched logistic regression consisted of terms for transfusion, caffeine, and furosemide. The relative

TABLE 3

Variables tested for association with necrotizing enterocolitis, Oklahoma City, Oklahoma, January–June 1985

Factor	Odds ratio	Chi-square	95% confidence interval
Transfusion	15.5	9.0430	2.6–92.5
Caffeine	5 7	3.7692	0.9–32.7
Furosemide	5.5	1.8837	0.5–62.8
Theophylline	4.5	1.4000	0.4–54.4
Indomethacin	2.7	0.4237	0.10–5.1
No stool (>3 days)	2 0	0.8205	0.5–8.9
Cesarean section	2.0	1.1164	0.6–7.2
Hospital of birth	1.9	1.6587	0 7–5.5
Gentamycin	1.7	0.4238	0.3–8.7
Early feeding (<48 hours)	1.6	0.4098	0.4–7.2
Hyperalimentation	1 6	0.4098	0.4–7.2
Prepartum infection	1.5	0.2462	0.3–8.4
Calcium supplement (IV)	1.5	0.3655	0.4–8.4
Hypoxic episode	1.0	0.0019	0.4–3.0
Ampicillin	1.0	0.0000	
Preeclampsia	0.9	0.3300	0.3–3 3
Ventilator	0.9	0.6274	0.3–2.6
Rectal stimulation	0.8	0.1480	0.2–2.9
Maternal diabetes	0.6	0.2727	0.1–4.7
Late feeding (>7 days)	0.6	0.5294	0.1–2.6
Umbilical artery catheter	0.5	1.5477	0.2–1.5
Umbilical venous catheter	0.4	1.1951	0.1–2.2
Exchange transfusion	0.000	1.0000	

risk of necrotizing enterocolitis in cases exposed to packed red blood cells transfusion compared with controls was 8.98 (95 per cent confidence interval = 1.08–74.6) when adjusted for the effects of caffeine and furosemide treatment.

DISCUSSION

The occurrence of an outbreak of necrotizing enterocolitis which involved a large number of infants provided an opportunity to examine the characteristics of this disease in its epidemic form and to search for factors related to its occurrence. Three important observations were made.

First, the birth weight-specific incidence of necrotizing enterocolitis in the outbreak setting appears similar to that observed in endemic necrotizing enterocolitis. In May and June, when clustering of cases was most evident, 90 per cent (18/20) of cases had birth weights less than 2,500 gm; for the entire outbreak period, the highest incidence occurred in the birth weight classes below 1,500 gm, with incidence rates in the other classes being similar in magnitude to one another. Previous studies have established that the incidence of the syndrome characteristically decreases as birth weight increases (2, 3, 9, 15). In the studies cited, birth weight-specific incidence was calculated using total live births in a given birth weight category as denominators; birth weight-specific incidence rates were calculated for this outbreak using the number of neonatal intensive care unit admissions as denominators. Thus, the approximate equality of incidence rates in the birth weight classes over 1,500 gm may result from an underrepresentation of heavier birth weight infants in admissions to the unit compared with the population of all liveborn infants. The predominant influence of birth weight (immaturity) in the development of the syndrome is apparent in the outbreak setting as well as in its endemic form.

Second, when the effect of birth weight

was taken into account by matching, no association of necrotizing enterocolitis and several exposures, previously reported as risk factors, was found in this study. Neither early feeding, use of umbilical catheters, nor exchange transfusion was associated with the syndrome in our data. These findings confirm results from recent studies of the endemic disease (2, 10, 15). In addition, hypoxic episodes which occurred during labor, at delivery, or immediately postpartum were not associated with this outbreak. The conclusion of no association must be considered in light of the power of the study. Evaluation of the power of these data to detect small, moderate, or large relative risks (2.0, 4.0, 8.0, respectively) revealed that for 1) use of umbilical catheters, and 2) hypoxic episodes, a risk of 8.0 or larger was reliably detectable (power = 0.722 and 0.722, respectively). However, a risk of 8.0 or less for the exposures, early feeding and exchange transfusion, was not reliably detectable (power = 0.464 and 0.03, respectively).

Third, transfusion of packed red blood cells was strongly and significantly associated with necrotizing enterocolitis, while use of drugs with diuretic properties was moderately associated. There are several possible ways in which transfusion of packed red blood cells may promote the development of the syndrome. The oxygen transport characteristics of stored red cells (16) and the presence in these cells of phthalate esters leached from polyvinylchloride plastic containers (17–19) have been identified as qualities of transfused cells that might promote ischemia. Polycythemia and hyperviscosity without polycythemia have experimentally been shown to decrease systemic and pulmonary blood flow (20, 21); in observational studies, polycythemia has been associated with necrotizing enterocolitis and gastrointestinal symptoms (22, 23). However, polycythemia was not characteristic of the cases studied, and the clinical significance of the altered oxygen transport characteristics of stored cells is doubtful (24, 25). The finding in the present study that diuretics and transfusion are associated with necrotizing enterocolitis raised the possibility that challenges to volume homeostasis, represented by these exposures, may promote development of the disease in the premature infant. This possibility deserves serious consideration in light of the study by Bell et al. (26, 27) showing an increased incidence of the disease among infants on high-volume fluid replacement therapy compared with those on low-volume replacement (26, 27).

In summary, the results of this investigation confirm the importance of birth weight, a host factor, in the development of necrotizing enterocolitis in the outbreak setting and suggest that volume homeostatic mechanisms may deserve attention in understanding the development of the syndrome.

References

1. Kliegman RM, Fanaroff AA. Neonatal necrotizing enterocolitis: a nine-year experience, I. epidemiology and uncommon observations. Am J Dis Child 1981;135:603–11.
2. Ryder RW, Shelton JD, Guinan ME, et al. Necrotizing enterocolitis: a prospective multicenter investigation. Am J Epidemiol 1980;112:113–23
3. Wilson R, Kanto WP Jr, McCarthy BJ, et al Epidemiologic characteristics of necrotizing enterocolitis: a population-based study. Am J Epidemiol 1981;114:880–7.
4. Santulli TV, Schullinger JN, Heird WC, et al. Acute necrotizing enterocolitis in infancy: a review of 64 cases. Pediatrics 1975;55:376–87.
5. Kliegman RM, Fanaroff AA. Necrotizing enterocolitis. N Engl J Med 1984;310:1093–1102.
6. Mizrahi A, Barlow O, Berdon W, et al. Necrotizing enterocolitis in premature infants. J Pediatr 1965;66:697–706.
7. Book LS, Overall JC, Herbst JJ, et al. Clustering of necrotizing enterocolitis: interruption by infection-control measures. N Engl J Med 1977,297: 984–6.
8. Brown EG, Sweet AY. Neonatal necrotizing enterocolitis. Pediatr Clin North Am 1982,29:1149–69.
9. Gaynes RP, Palmer J, Martone WJ, et al. The role of host factors in an outbreak of necrotizing enterocolitis. Am J Dis Child 1984;138:1118–20
10. Yu VYH, Joseph R, Bajuk B, et al. Perinatal risk factors for necrotizing enterocolitis. Arch Dis Child 1984;59:430–4.
11. Thilo EH, Lazarte RA, Hernandez JA. Necrotizing enterocolitis in the first 24 hours of life. Pediatrics 1984;73:476–80.
12. Schlesselman JJ. Case-control studies—design, conduct, analysis. New York: Oxford University

Press, 1982 160–3, 214–20
13. Kleinbaum DG, Kupper LL, Morgenstern J. Epidemiologic research: principles and quantitative methods. Belmont, CA: Lifetime Learning Publications, 1982:372–4, 419–56.
14. Lubin JH. A computer program for the analysis of matched case-control studies. Comput Biomed Res 1981;14:138–43.
15. Stoll BJ, Kanto WP Jr, Glass RI, et al Epidemiology of necrotizing enterocolitis: a case-control study. J Pediatr 1980;96·447–51.
16. Stockman JA. The anemia of prematurity and the decision when to transfuse. Pediatr Clin North Am 1984;33.111–28.
17. Lang DJ, Valeri CR. Hazards of blood transfusion. Adv Pediatr 1977;24:311–38.
18. Contreras TJ, Sheibley RH, Valeri CR. Accumulation of di-2-ethylhexylphthalate (DEHP) in whole blood, platelet concentrate, and platelet-poor plasma. Transfusion 1974;14:34–46.
19 Hillman LS, Goodwin SL, Sherman WR. Identification and measurement of plasticizer in neonatal tissues after umbilical catheters and blood products. N Engl J Med 1975;292·381–6.
20. Fouron J, Hebert F. The circulatory effects of hematocrit variations in normovolemic newborn lambs. J Pediatr 1973;82:995–1003.
21. Holzman IR, Tabata B, Edelstone DI. Effects of varying hematocrit on intestinal oxygen uptake in neonatal lambs. Am J Physiol 1985:248:G432–6.
22. Black VD, Lubchenco LD. Neonatal polycythemia and hyperviscosity. Pediatr Clin North Am 1982;29:1137–49
23. Black VD, Rumack CM, Lubchenco LD, et al. Gastrointestinal injury in polycythemic term infants. Pediatrics 1985;76:225–31.
24. Stockman JA, Clark DA. Weight gain: a response to transfusion in selected preterm infants. Am J Dis Child 1984;138:828–30.
25. Blank JP, Sheagran TG, Jayshree V, et al The role of RBC transfusion in the premature infant. Am J Dis Child 1984;138:831–3.
26 Bell EF, Warburton D, Stonestreet BS, et al Effect of fluid administration on the development of symptomatic patent ductus arteriosus and congestive heart failure in premature infants N Engl J Med 1980;802:598–604.
27 Bell RF, Warburton D, Stonestreet BS, et al. High volume fluid intake predisposes premature infants to necrotizing enterocolitis. (Letter.) Lancet 1979:2:90.