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Risk factors of necrotizing enterocolitis in neonates with sepsis: A retrospective case-control study

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Abstract

Sepsis, a severe infectious disease in the neonatal period, is considered a risk factor for necrotizing enterocolitis (NEC). To investigate the specific risk factors for NEC in septic infants, septic infants admitted to our center from January 2010 to April 2018 were included. Septic neonates with proven NEC (Bell's stage ⩾II) were enrolled in the NEC group, and those without NEC were enrolled in the control group. Demographics, clinical characteristics, and risk factors were compared between the two groups. Univariate and logistic regression analyses were used to evaluate the potential risk factors for NEC. A total of 610 septic neonates were included, of whom 78 (12.8%) had complicated NEC. The univariate analysis indicated that infants with NEC had a lower birth weight, a lower gestational age, and older age on admission than those without NEC ($P < 0.05$). Higher rates of anemia, prolonged rupture of membranes (PROM) (⩾18 h), pregnancy-induced hypertension, late-onset sepsis (LOS), red blood cell transfusion and hypoalbuminemia were observed in the NEC group than in the non-NEC group ($P<0.05$). Logistic regression analysis revealed LOS ($P = 0.000$), red blood cell transfusion ($P = 0.001$) and hypoalbuminemia ($P = 0.001$) were associated with the development of NEC. Among NEC infants, those who needed red blood cell transfusion had a longer hospitalization duration than those who did not need transfusion ($P < 0.05$). LOS, red blood cell transfusion and hypoalbuminemia were independent risk factors for the development of NEC in infants with sepsis. Taking measures to reduce the occurrence of hypoproteinemia and severe anemia may help to reduce the occurrence of NEC in septic neonates.

Keywords

hypoalbuminemia, late-onset sepsis, necrotizing enterocolitis, red blood cell transfusion, risk factors

Introduction

Neonatal necrotizing enterocolitis (NEC) is a serious gastrointestinal disease in neonates. The incidence of NEC is 7‰–13‰, and the mortality rate of NEC ranges from 20% to 30%.[1,2] Survivors often experience short-term and long-term complications, such as intestinal stenosis, short bowel syndrome and neurological sequelae.[3–5] Although 90% of infants who develop NEC are born prematurely, full-term and near-term infants can also develop the disease. Although the etiology of NEC

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remains unclear, multiple risk factors, such as prematurity, low birth weight, hypoxia, abnormal microbiota colonization in the intestinal tract, microcirculatory disorders, formula feeding and patent ductus arteriosus, are involved in the development of NEC.[6,7] Although NEC predominately affects premature infants, approximately 10% of NEC cases are diagnosed in late-preterm and full-term infants. Late preterm and full-term infants are also more likely to develop NEC if they have other risk factors, such as cyanotic congenital heart disease, intrauterine growth retardation, exchange transfusions, polycythemia and maternal illicit drug use.[8] Sepsis as a severe infectious disease and is considered a risk factor for NEC.[9] The incidence of NEC in sepsis patients ranges from 34% to 57%.[10,11] Therefore, identifying specific risk factors for NEC in infants with sepsis will be helpful to optimize strategies to reduce morbidity and mortality. This study aims to analyze the risk factors for the development of NEC in infants with sepsis to provide new directions for clinical treatment strategies.

Materials and methods

Setting

Our center is a national clinical specialty department, which has 300 beds and admits approximately 10,000 newborns each year.

Clinical data collection

Septic infants admitted to the Children's Hospital of Chongqing Medical University Neonatal Department from January 2010 to April 2018 were included in the present study. All septic infants were included in the study. Those who subsequently developed proven NEC (Bell's stage$\geqslant$II) were enrolled in the NEC group, and the others without NEC were enrolled in the control group. Infants with incomplete information or with NEC prior to sepsis were excluded from the analysis. Cases were excluded if the newborn had any symptoms including abdominal distention, vomiting, bloody stool, diarrhea or feeding intolerance at the time of sepsis diagnosis. Clinical data were obtained from the electronic medical record system of Chongqing Children's Hospital. Maternal and neonatal demographic, comorbidities or complication, laboratory examination, treatment protocol and clinical outcome data were collected. The study was approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University (Approval No. 2016-17) and use of the database housing the evaluated data was permitted by the ethics committees of CHCMU. The Ethics Committee waived the requirement for informed consent due to the anonymized nature of the data and scientific purpose of the study.

Definitions

Culture-proven sepsis was diagnosed when a pathogen was isolated from blood or cerebrospinal fluid and the infants with infectious manifestations were treated with antibiotics for $\geqslant$5 days. When coagulase-negative staphylococcal species (CoNS) were isolated from blood, definite sepsis was diagnosed either when there were 2 time-separated cultures of the same species and the infant had been treated with antibiotics for $\geqslant$5 days or when a single CoNS species was isolated in association with abnormal blood markers of sepsis (white blood cells (WBCs) $<5\times10^9$ or $>20\times10^9$/l, C-reactive protein (CRP) $>$10 mg/l, immature/total neutrophil (I/T) ratio $>$0.12, platelets $<$100,000/mm^3), and treatment with $\geqslant$5 days of antibiotics. When blood or cerebrospinal fluid culture was negative, the clinical diagnosis of sepsis was based on the presence of three or more of the following criteria: (1) antenatal risk factors (prolonged rupture of membranes (PROM) $>$18 h, chorioamnionitis or positive evidence of group B streptococcal disease (GBS)); (2) clinical signs including respiratory dysfunction (distress or apnea), tachycardia (heart rate $>$190 beats/min) or bradycardia (heart rate $<$90 beats/min), cardiovascular compromise (e.g. paleness or peripheral cyanosis and mottled skin with capillary refill delayed $>$3 s), and neurological signs (seizures, irritability, lethargy); and (3) positive results on conventional laboratory tests (WBCs $<5\times10^9$ or $>20\times10^9$/l, CRP $>$10 mg/l, I/T ratio $>$0.12, platelets $<$100,000/mm^3).[12] Early-onset sepsis (EOS) was defined as infection occurring less than 72 h after birth, and late-onset sepsis (LOS) was diagnosed based on the age at onset, with bacteremia or bacterial meningitis occurring at $>$72 h.[1] NEC was defined as the presence of one or more of the parenthesized clinical signs, including drowsiness, unstable body temperature,

apnea, bradycardia, vomiting, bloating, bloody stool, and at least one of the following three radiographic or sonographic findings: pneumatosis intestinalis, portal vein gas and/or pneumoperitoneum.[13] Those with digestive tract malformations such as congenital ileum, Hirschsprung's disease and congenital intestinal malrotation were excluded. Anemia was defined as a central venous hematocrit $<39\%$.[14] Hypoalbuminemia was defined as serum albumin <25 g/L.[15] Sclerema neonatorum was defined as a type of panniculitis involving hardening of the skin and subcutaneous adipose tissue.[16] Birth asphyxia was defined as the failure to initiate or sustain spontaneous breathing at birth, a 1-min Apgar score <7 and cord umbilical arterial pH <7.15. Small for gestational age was defined as a birth weight and/or length below the mean for gestational age (<2 SD).[17] The diagnosis of meconium-stained amniotic fluid was based on the passage of fetal colonic contents into the amniotic cavity.[18] Simple congenital heart defect (CHD) was defined as an isolated and uncomplicated secundum atrial septal defect (ASD), patent ductus arteriosus (PDA), ventricular septal defect (VSD) with normal pulmonary vascular resistance, or mild pulmonary stenosis (PS) verified by two-dimensional ultrasonography.[19] Complex CHD was defined as defects requiring surgery before 12 months of age.[20] The duration between sepsis and the onset of NEC was defined as the time from sepsis diagnosis to the onset of NEC. The duration between red blood cell transfusion before NEC and the onset of NEC was defined as the time from last blood transfusion to NEC onset.

statistical analysis

All data were analyzed by SPSS 13.0 (SPSS Inc. Chicago, IL). The normality of the distribution of continuous variables was evaluated by the Kolmogorov-Smirnov test, and comparisons were analyzed using Student's *t*-test. Nonnormally distributed data are expressed as medians and interquartile ranges and were compared by the Mann-Whitney *U* test. Categorical variables were tested using the chi-square test or Fisher's exact test. Multivariate logistic regression was performed to identify independent risk factors for the development of NEC in septic infants. Statistical significance was established at $P < 0.05$.

Results

Clinical characteristics of the septic infants

During the study period, 610 septic infants were admitted to the Children's Hospital of Chongqing Medical University; 78 (12.8%) developed NEC and were enrolled in the NEC group.

These newborns were diagnosed with NEC at an average age of 12 (5.24–22.1) days. The duration between sepsis and the onset of NEC was 5 (3–8) days. The duration of red blood cell transfusion before NEC and the onset of NEC was 24 (12–48) h, and the duration of albumin transfusion before NEC and the onset of NEC was 4 (1.5–7) days. Fourteen (17.9%) septic infants with NEC and 128 (24.1%) septic infants without NEC were positive on blood culture ($\chi^2 = 1.423$, $P > 0.05$).

Table 1 shows that infants who finally developed NEC had a lower gestational age, a lower birth weight, older age on admission and a higher rate of LOS than those who did not ($P < 0.05$). The rate of PROM and pregnancy-induced hypertension (PIH) was higher in the NEC group than in the non-NEC group ($P < 0.05$). No differences in demographic features in septic infants, including sex, cesarean section, small for gestational age, meconium-stained amniotic fluid, antenatal corticosteroid use, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, chorioamnionitis, syphilis during pregnancy, anemia during pregnancy, perinatal asphyxia and feeding mode, were found between the two groups ($P > 0.05$).

Risk factors for NEC in infants with sepsis according to univariate analysis

Table 2 shows the differences in neonatal comorbidities before the onset of NEC and treatment strategies between the two groups. The NEC group had higher rates of anemia, hypoalbuminemia, sclerema neonatorum and red blood cell transfusion than the non-NEC group ($P < 0.05$). No differences were found in meconium aspiration syndrome, apnea, respiratory failure or pulmonary hemorrhage between the two groups ($P > 0.05$). The rates of breastfeeding, mechanical ventilation, and probiotic use were also not significantly different between the groups ($P > 0.05$).

Blood culture tests were performed in all infants, and positive cultures were obtained in 142 infants. The positive rate of blood culture was not different

Table 1. Demographic characteristics of septic infants.

Variables	With NEC (n = 78)	Without NEC (n = 532)	Statistics	*P*
	Mean ± SD, M (P_{25}–P_{75}), n (%)			
Male	47 (60.26)	333 (62.59)	$\chi^2 = 0.158$	0.691
Gestational age, weeks	35.88 ± 3.60	37.11 ± 3.73	$t = 2.734$	0.006
Birth weight, g	2469.46 ± 903.64	2732.09 ± 868.54	$t = 409$	0.018
Cesarean section	43 (55.13)	287 (53.95)	$\chi^2 = 038$	0.845
Small for gestational age	9 (11.54)	36 (6.77)	$\chi^2 = 2.267$	0.132
Age on admission, days	3.98 (0.98–14.11)	1.22 (0.21–9.47)	$Z = 3.267$	0.001
Late-onset sepsis	49 (62.8)	206 (38.7)	$\chi^2 = 16.239$	0.000
Positive blood culture	14 (17.95)	128 (24.06)	$\chi^2 = 1.423$	0.233
Prolonged rupture of membranes, ⩾18 h	18 (23.08)	64 (12.03)	$\chi^2 = 7.135$	0.008
Pregnancy-induced hypertension	10 (12.82)	25 (4.70)	$\chi^2 = 6.862$[a]	0.009
Meconium-stained amniotic fluid	13 (16.67)	115 (21.62)	$\chi^2 = 1.005$	0.316
Antenatal corticosteroid use	6 (7.70)	30 (5.64)	$\chi^2 = 0.213$[a]	0.645
Gestational diabetes mellitus	2 (2.56)	38 (7.14)	$\chi^2 = 2.328$	0.127
Intrahepatic cholestasis of pregnancy	1 (1.28)	7 (1.31)	$\chi^2 = 0.000$	1
Chorioamnionitis	0 (0)	3 (0.56%)	—	1[b]
Syphilis during pregnancy	1 (1.28)	10 (1.88)	$\chi^2 = 0.000$[a]	1
Anemia during pregnancy	12 (15.38)	89 (16.73)	$\chi^2 = 0.089$	0.765
Perinatal asphyxia	7 (8.97)	51 (9.59)	$\chi^2 = 0.03$	0.863
Feeding mode				
Breastfeeding	8 (10.26)	71 (13.34)	$\chi^2 = 7.342$	0.062
Formula feeding	31 (39.74)	135 (25.38)		
Mixed feeding	9 (11.54)	88 (16.54)		
No enteral feeding	30 (38.46)	238 (44.74)		

[a]Correct chi-square value.
[b]Fisher's exact value.

Table 2. Univariate analysis of risk factors for NEC onset in septic infants, n (%).

Variables	NEC (n = 78)	Non-NEC (n = 532)	χ^2	*P*
Comorbidity before NEC onset				
Meconium aspiration syndrome	1 (1.3)	6 (1.1)	—	1[b]
Respiratory failure	22 (28.2)	182 (34.2)	1.102	0.294
Septic shock	2 (2.6)	6 (1.1)	0.258	0.611[a]
Simple congenital heart disease	17 (21.8)	140 (26.3)	0.727	0.394
Complex congenital heart disease	0	2 (0.4)	—	1[b]
ABO hemolytic disease	4 (5.1)	34 (6.4)	0.032	0.857
Heart failure	2 (2.6)	3 (0.6)	—	0.125[b]
Sclerema neonatorum	10 (12.8)	35 (6.6)	3.878	0.049
Apnea	4 (5.1)	31 (5.8)	0.000	1[a]
Pulmonary hemorrhage	7 (9.0)	51 (9.6)	0.03	0.863
Anemia	54 (69.2)	204 (38.3)	26.587	0.000
Hypoalbuminemia	45 (57.7)	170 (32.0)	19.744	0.000
Treatment before NEC onset				
Breast feeding	8 (10.3)	71 (13.3)	0.576	0.448
Mechanical ventilation	23 (29.5)	185 (34.8)	0.846	0.358
Red blood cell transfusion	39 (50.0)	123 (23.1)	25.199	0.000
Probiotic use	36 (46.2)	218 (41.0)	0.75	0.386

[a]Correct chi-square value.
[b]Fisher's exact value.

Table 3. Distribution of pathogens in blood cultures.

Bacteria	NEC (n = 78)	Non-NEC (n = 532)	χ^2	*P*
CoNS*, n (%)	2 (2.6)	23 (4.3)	0.182	0.67[a]
Gram-negative bacilli, n (%)	7 (9.0)	71 (13.3)	1.166	0.28
Fungi, n (%)	3 (3.8)	17 (3.2)	0.000	1[a]
Streptococcus lactis, n (%)	0	5 (0.9)	—	1[b]
Listeria monocytogenes, n (%)	0	2 (0.4)	—	1[b]
Others, n (%)	2 (2.6)	10 (1.9)	0.000	1[a]

*CONS: coagulase-negative staphylococcus.
[a]Correct chi-square value.
[b]Fisher's exact value.

between the NEC group and the non-NEC group (17.95% (14/78) vs 24.06 (128/532), $\chi^2 = 1.423$, $P = 0.233$). As shown in Table 3, gram-negative bacilli were the main pathogens in positive blood cultures, followed by CoNS and fungal pathogens. There was no significant difference in bacterial distribution between the NEC group and the non-NEC group.

Stepwise logistic regression analysis for risk factors for NEC onset in infants with sepsis

All parameters with $P < 0.05$ in the univariate analyses, including gestational age, birth weight, LOS, PROM, PIH, sclerema neonatorum, hypoalbuminemia, and red blood cell transfusion, were included in the multivariate analysis. LOS, red blood cell transfusion and hypoalbuminemia were considered independent risk factors for the development of NEC in septic infants (Table 4).

Comparison of features between NEC infants with or without blood transfusion

As shown in Table 5, the transfusion group had a lower birth weight, lower gestational age, higher frequency of antenatal corticosteroid exposure and longer hospitalization duration than the non-transfusion group ($P < 0.05$). There were no significant differences in the rates of LOS, positive blood culture, maternal factors, meconium-stained amniotic fluid, stage III NEC or mortality ($P > 0.05$).

Discussion

Sepsis has been identified as a risk factor for NEC in several studies.[21,22] Studies have shown that the incidence of NEC in infants with sepsis is almost three-fold that in infants without sepsis.[11] In the present study, we found that 12.8% of septic infants developed NEC, and LOS significantly increased the incidence of NEC; this finding was similar to other studies.[23,24] Thus, investigating the specific risk factors for NEC in septic infants may be helpful to decrease the morbidity and mortality of NEC in infants with sepsis.

It is estimated that 25~35% of NEC cases are associated with red blood cell transfusion.[25] In our study, red blood cell transfusion was an independent risk factor for the development of NEC and 50% of the patients who finally developed NEC received 1~6 red blood cell transfusions before NEC, while 23.1% of cases without NEC received red blood cell transfusion. The duration between red blood cell transfusion before NEC and the onset of NEC was 24 (12–48) h. These results were similar to those in Edlib's report,[26] in which the duration was closer to 48–72 h. As shown in Table 5, the lower the birth weight and gestational weeks were, the higher the risk of blood transfusion was. Anemia is a common complication of sepsis, and in our study, 26.6% (162/610) of all septic cases received red blood cell transfusion. However, red blood cell transfusion was identified as a predictor of NEC onset among all septic infants. One potential mechanism may be that anemia induces reduced mesenteric blood flow leading to intestinal hypoxia, and subsequently, ischemia-reperfusion leads to bowel injury caused by red blood cell transfusion.[27–29] Another potential mechanism may be that red blood cell transfusion may increase some proinflammatory cytokines, such as IL-1β, IL-8, and IFN-γ, which may increase the local inflammatory responses and cause NEC.[30]

Hypoalbuminemia was another independent risk factor for the development of NEC in septic infants in this study, and the duration of albumin

Table 4. Multivariate analysis of predictors of NEC onset in septic infants.

Variables	β	SE	Wald	*P*	OR	95% CI
Late-onset sepsis	0.918	0.259	12.603	0.000	2.505	1.509–4.16
Red blood cell transfusion	0.902	0.264	11.712	0.001	2.466	1.471–4.134
Hypoalbuminemia	0.867	0.262	10.955	0.001	2.38	1.424–3.978
Constant	−4.861	0.518	87.924	0.000	—	—

Table 5. Comparison of features between NEC infants with or without blood transfusion.

Variables	Transfusion group (n = 39)	Nontransfusion group (n = 39)	$T/Z/\chi^2$	*P*
	Mean ± SD, M (P_{25}–P_{75}), n (%)			
Gestational age, weeks	34.30 ± 3.79	37.46 ± 2.6	4.286	0.000
Birth weight, g	2069.33 ± 803.38	2869.59 ± 3.79	4.34	0.000
Age at admission, days	3.19 (0.35–19.42)	5.24 (2.39–12.12)	0.555	0.579
Late-onset sepsis	28 (71.8)	21 (53.8)	2.69	0.101
Positive blood culture	9 (23.1)	5 (12.8)	1.393	0.238
Prolonged rupture of membranes, ⩾18 h	10 (25.6)	8 (20.5)	0.239	0.591
Gestational diabetes mellitus	1 (2.6)	1 (2.6)	0.000	1
Pregnancy-induced hypertension	6 (15.4)	4 (10.3)	0.459	0.498
Male	26 (66.7)	21 (53.8)	1.338	0.247
Cesarean section	24 (61.54)	19 (48.72)	1.296	0.225
Meconium-stained amniotic fluid	23.1 (9)	10.3 (4)	2.308	0.129
Antenatal corticosteroid use	15.4 (6)	0	4.514	0.034
Small for gestational age	5 (12.8)	4 (10.3)	0.000	1
NEC stage III	5 (12.8)	2 (5.1)	0.628	0.428
Hospitalization duration	34.46 ± 24.93	16.56 ± 9.4	4.19	0.000
Mortality	11 (28.2)	13 (33.3)	0.241	0.624

transfusion before NEC and the onset of NEC was 4 (1.5–7) days. Hypoalbuminemia is a common complication of sepsis, and the level of serum albumin might be reduced by approximately 10-15 g/L within 1 week of the event. Inflammatory mediators such as IL-1, IL-6, and TNF-α can decrease albumin synthesis.[15] Oxidative stress has been proven to be involved in the pathogenesis of NEC,[31] and albumin implicates in the antioxidant capacity of plasma.[32,33] Thus, hypoproteinemia may decrease the plasma antioxidant capacity, resulting in the deterioration of NEC. Therefore, an aggressive strategy for preventing hypoalbuminemia in septic infants might reduce the occurrence of NEC.[34]

Our findings highlight that improving medical measures to reduce the incidence of anemia and hypoproteinemia may help reduce the incidence of NEC in septic neonates. The present study also has some limitations, including the errors and bias inherent to the nature of the retrospective design. Breast milk is the protective factor of NEC, and most infants were formula fed during hospitalization in the present study, which might have increased the incidence of NEC.[35] Moreover, we did not identify an association between anemia degree and the severity of NEC in infants with sepsis due to the limited number of samples at our single center; in the future, multicenter, large-sample studies are recommended.

In a word, our study found that red blood cell transfusion and hypoalbuminemia were risk factors for the development of NEC in septic infants. Improving medical measures to reduce the incidence of anemia and hypoproteinemia may help reduce the incidence of NEC in septic neonates.

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Ethical approval

Ethical approval for this study was obtained from the Ethics Committee of the Children's Hospital of Chongqing Medical University (APPROVAL NUMBER/ID:2016-17).

Informed consent

Informed consent was not sought for the present study because it is a retrospective clinical study.

Trial registration

Not applicable.

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