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Broad-spectrum Antibiotic Plus Metronidazole May Not Prevent the Deterioration of Necrotizing Enterocolitis From Stage II to III in Full-term and Near-term Infants

A Propensity Score-matched Cohort Study

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Abstract: Necrotizing enterocolitis (NEC) is the most common and frequently dangerous neonatal gastrointestinal disease. Studies have shown broad-spectrum antibiotics plus anaerobic antimicrobial therapy did not prevent the deterioration of NEC among very low birth preterm infants. However, few studies about this therapy which focused on full-term and near-term infant with NEC has been reported. The aim of this study was to evaluate the effect of broad-spectrum antibiotic plus metronidazole in preventing the deterioration of NEC from stage II to III in full-term and near-term infants.

A retrospective cohort study based on the propensity score (PS) 1:1 matching was performed among the full-term and near-term infants with NEC (Bell stage ≥II). All infants who received broad-spectrum antibiotics were divided into 2 groups: group with metronidazole treatment (metronidazole was used ≥4 days continuously, 15 mg/kg/day) and group without metronidazole treatment. The depraved rates of stage II NEC between the 2 groups were compared. Meanwhile, the risk factors associated with the deterioration of stage II NEC were analyzed by case-control study in the PS-matched cases.

A total of 229 infants met the inclusion criteria. Before PS-matching, we found the deterioration of NEC rate in the group with metronidazole treatment was higher than that in the group without metronidazole treatment (18.1% [28/155] vs 8.1% [6/74]; $P = 0.048$). After PS-matching, 73 pairs were matched, and the depraved rate of NEC in the group with metronidazole treatment was not lower than that in the group without metronidazole treatment (15.1% vs 8.2%; $P = 0.2$). Binary logistic regression analysis showed that sepsis after NEC (odds ratio [OR] 3.748, 95% confidence interval [CI] 1.171–11.998, $P = 0.03$), the need to use transfusion of blood products after diagnosis of NEC (OR 8.003, 95% CI 2.365–27.087, $P = 0.00$), and the need of longer time for nasogastric suction were risk factors for stage II NEC progressing to stage III (OR 1.102, 95% CI 1.004–1.21, $P = 0.04$).

Broad-spectrum antibiotic plus metronidazole may not prevent the deterioration of NEC in full-term and near-term infants. Those infants who had sepsis required transfusion of blood products, and needed longer time for nasogastric suction after stage II NEC was more likely to progress to stage III.

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Abbreviations: CRP = C-reactive protein, IL-17 = interleukin 17, IQR = interquartile range, NEC = necrotizing enterocolitis, PS = propensity score.

INTRODUCTION

Necrotizing enterocolitis (NEC) is one of the most devastating gastrointestinal inflammatory diseases in neonatal intensive care units,[1] and the mortality associated with NEC ranges from 20% to 30%.[2] Compared with infants with stage II NEC, higher mortality rate, higher costs of hospitalization and care, and more significant sequelae were common in those with stage III NEC.[3] For stage III NEC cases, some of them occurred as stage III at disease onset, whereas others developed from stage II.[3] For the infants with stage II NEC, they were more likely to progress to stage III if they were of earlier gestational age, lower birth weight, lacking early colostrums feedings, or with an elevated C-reactive protein (CRP) or sepsis.[3,4] Some of the above risk factors (such as low gestational age and birth weight) could not be changed once NEC was diagnosed. However, the sepsis variable could be influenced, to a certain extent, by the neonatal caregivers. Certainly, any efforts trying to reduce the onset of sepsis may actually decrease the severity of NEC. Meanwhile, these studies did not focus on the efficiency of antibiotics,[3,4] which was strongly recommended for NEC management.[2,5,6] Thus, it is worthy of studying the relationship of different antibiotic strategies with the progress and the severity of NEC. In NEC treatment, only broad-spectrum antibiotics[2] or broad-spectrum antibiotics plus anaerobic antimicrobial therapy were recommended.[7,8] Faix et al[8] and Shah[9] found broad-spectrum antibiotics plus anaerobic antimicrobial therapy was of no benefit on the mortality of NEC. Moreover, Autmizguine et al[7] found broad-spectrum antibiotics plus anaerobic antimicrobial therapy did not prevent NEC progressing from stage II to III among infants having birth weight less than 1500 g and among preterm infants. Full-term or near-term infants have relatively mature intestinal and immune systems, and this makes NEC fundamentally different in features between full-term, near-term, and preterm infants.[10–12] Compared with preterm infants, NEC

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occurred earlier in full-term and near-term infants,[11–13] and the diversity of the intestinal bacterial community in full-term and near-term infants was different from that in preterm neonates at the onset of NEC.[13] Thus, the effect of broad-spectrum antibiotics plus anaerobic antimicrobial therapy on preterm infants might not reflect its profile on full-term and near-term patients. Though NEC is more common in early preterm infants (gestation age less than 34 weeks), it is estimated that 10% to 15% of NEC cases occur in full-term infants.[12,14] According to the systematic review published in Cochrane Database Syst Rev in 2012,[9] to our current knowledge, few studies about this therapy among full-term and near-term infants has been reported. The aim of this study was to evaluate the effect of broad-spectrum antibiotic plus metronidazole therapy on preventing the deterioration of NEC from stage II to stage III in full-term and near-term infants.

METHODS

Data Collection

The study was approved by the Institutional Review Board of the Children's Hospital of Chongqing Medical University (Approval No. 119/2014). A retrospective cohort study was conducted. Medical records were reviewed for full-term (gestation age ≥37 weeks) and near-term infants (gestation age ≥34 weeks) with NEC (Bell stage ≥II),[15] admitted to the Children's Hospital of Chongqing Medical University from January 2008 to March 2015.

The stage of NEC was diagnosed according to the criteria originally proposed by Bell et al, subsequently modified by Walsh and Kliegman.[15] The stage II NEC was defined according to the following 2 criteria: presence of clinical signs such as abdominal distension and emesis or gross blood in the stool (with an absence of fissure); and having radiographic or ultrasound findings of pneumatosis intestinalis or portal vein gas. The stage III NEC criteria included the above plus radiographic or ultrasound findings of the pneumoperitoneum or large amounts of ascites, or someone who requires bowel surgery if medical therapy had no effect within 48 hours.[3,15]

The age of NEC onset was defined as the day in which at least 1 of the following signs or symptoms appeared: prefeeding gastric residuals, emesis, abdominal distension, or bloody stool. The age of NEC diagnosis was defined as the day of the abdominal X-ray or the ultrasound findings meeting the diagnostic criteria of NEC. The diagnosis of NEC was made, a sepsis evaluation was done promptly, and sepsis after NEC was defined as it occurred more than 24 hours after the diagnosis of NEC. The patients with spontaneous intestinal perforation, intestinal malformation (aproctia, intestinal atresia, Hirschsprung disease), or with stage III NEC at the onset of the disease were excluded from the study. The patients with incomplete information were also excluded from the present study. Once the NEC case was identified and included in the present study, all physicians' and nurses' notes pertaining to NEC, laboratory examinations, radiographic and ultrasonic reports, and surgical records were reviewed.

All patients included in this study underwent basically the same protocol of treatment including cessation of enteral feeding, total parenteral nutrition, nasogastric suction, intensive care therapy (cardiorespiratory support and blood or blood products transfusion) if necessary. All infants received one of the following broad-spectrum antibiotics (average 14.67 days), including semisynthetic penicillins, cephalosporin, carbapenems, vancomycin, and so on. More than 3 days of antibiotic therapy was recommended for stage ≥II NEC,[16,17] and we speculated that using antianaerobic regimen less than 3 days might not have sufficient efficacy for stage ≥II NEC. Therefore, we divided the patients into 2 groups: the group with metronidazole treatment (metronidazole was used ≥4 days continuously, 15 mg/kg/day) and the group without metronidazole treatment (metronidazole was not used).

Statistical Analysis

As multiple factors could affect the severity of NEC[3,4] and metronidazole was not used in a randomized manner in our study, to avoid attributing to metronidazole for the incidence of advanced stage of NEC what may be attributable to other factors, we established a propensity score (PS) for metronidazole (with or without).[18] This score was obtained with a logistic regression model that included the antenatal information, demographic data, complications, and treatment protocol of infants. Then, we performed 1:1 matching by using the nearest neighbor without replacement, and it was allowed only if the difference in PS between the group without metronidazole treatment and the group with metronidazole treatment was <0.02.[19] In the PS-matched cohort, normally distributed continuous data were described as mean ± standard deviation (M ± SD) and were analyzed using an independent 2-tailed *t* test. Skewed data were described as median (interquartile range [IQR]) and analyzed by Mann–Whitney *U* tests. Categorical data were analyzed by the chi-square tests, or Fisher exact test. The data were processed with SPSS13.0 (SPSS Inc., Chicago, IL) using descriptive and inferential statistics. Statistical significance was established if $P < 0.05$.

RESULTS

Study Population

A total of 229 infants met the inclusion criteria for further study, after excluding 80 infants not meeting the criteria (21 spontaneous intestinal perforation or intestinal malformation, 12 infants used 1–3 days of metronidazole, 30 infants with stage III at the onset of NEC, 17 incomplete information). The demographic features of infants are shown in Table 1. The complications and treatment protocol of infants were shown in Tables 2 and 3, respectively. Overall, the median gestational age was 38 weeks, approximately 60% were males, only about 20% were fed by breast milk before NEC, the age of onset was approximately 6 days after birth, and 11.3% (34/229) of the included infants progressed to stage III during hospitalization (Table 1).

Before PS-matching, the demographic features were similar between the group with metronidazole treatment (n = 155) and the group without metronidazole treatment (n = 74). With exceptions to coagulopathy ($P = 0.01$), vaginal delivery ($P = 0.03$), and male ($P = 0.02$), all other main complications and treatment protocols exhibited no significant difference between the 2 groups (Tables 2 and 3).

After PS-matching, 73 infants from the group with metronidazole treatment were matched to 73 infants from the group without metronidazole treatment, yielding a final cohort of 146 infants. The demographic features such as gestation age, birth weight, the age of onset NEC, the main complications, and the treatment protocol (Tables 1–3) still had no significant difference in the 2 groups. The 2 variables of coagulopathy and vaginal delivery turned to be comparable between the 2 groups after PS-matching, and the statistical difference of gender variable between the 2 groups decreased. Thus, the PS-matching provided a better balanced cohort between the 2 groups in the present study.

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TABLE 1. The Features of NEC Infants Treated by Broad-spectrum Antibiotics Plus (With/Without) Metronidazole in This Study

		Before PS-matching (% [n])			After PS-matching (% [n])		
	Total Feature	Without (n = 74)	With (n = 155)	*P*	Without (n = 73)	With (n = 73)	*P*
PROM, % (n)	3.4 (8)	5.4 (4)	2.6 (4)	0.48	5.5 (4)	2.7 (2)	0.68
Amniotic fluid contamination,% (n)	10 (23)	5.4 (4)	12.3 (19)	0.11	5.5(4)	12.3 (9)	0.15
Asphyxia, % (n)	3.9 (9)	4.1 (3)	3.9 (6)	1	4.1 (3)	1.4 (1)	0.61
PIH, % (n)	2 (12)	2.7 (2)	6.5 (10)	0.38	2.7 (2)	5.5 (4)	0.68
ICP, % (n)	1.3 (3)	0	1.9 (3)	0.55	0	1.4 (1)	1
IDM, % (n)	3 (7)	0	4.5 (7)	0.15	0	5.5 (4)	0.13
Vaginal delivery, % (n)	34.5 (79)	44.6(33)	29.7 (46)	0.03	43.8 (32)	37 (27)	0.4
Gender (male), % (n)	61.1 (140)	50 (37)	66.5 (103)	0.02	49.3 (36)	65.8 (48)	0.05
Gestational age, IQR, wks	38.71 (37–39.71)	38.86 (37–39.71)	38.57 (37–39.71)	0.64	38.86 (37.14–39.71)	38.86 (37.43–39.71)	0.71
Birth weight (±SD), g	2940.4 ± 595.3	2887.8 ± 517.9	2965.5+628.9	0.36	2905.39+498.6	2914.2 + 500	0.92
Multiple gestations, % (n)	6.1 (14)	5.4 (4)	6.5 (10)	0.99	5.5 (4)	5.5 (4)	1
Breastfed, % (n)	21.8 (50)	17.6 (13)	23.9 (37)	0.11	17.8 (13)	26 (19)	0.17
The age of onset, IQR, d	6 (1–12)	6 (1–11)	6 (1–12)	0.85	6 (1–11)	7 (2–13)	0.29
The age of diagnosis, IQR, d	8 (2–17)	8.63 (2.83–17)	8 (2–17)	0.82	8.25 (2.67–17)	11 (3.5–18)	0.49

ICP = intrahepatic cholestasis of pregnancy, IDM = infants of diabetic mother, NEC = necrotizing enterocolitis, PIH = pregnancy-induced hypertension, PROM = prolonged rupture of membranes > 18 h.

The Influence of the Therapy on the Deterioration of NEC Between the 2 Groups

Before PS-matching, we found the deterioration of NEC rate in the group with metronidazole treatment was higher than that in the group without metronidazole treatment (18.1% [28/155] vs 8.1% [6/74]; $P = 0.048$), and the mortality between the group with and the group without metronidazole treatments had no significant difference (21.4% [6/28] vs 33.3% [2/6]; $P = 0.93$) among infants in stage III group (n = 34). After PS-matching, we found that the rate for NEC deteriorating from stage II to III in the group with metronidazole treatment was not lower than that in the group without metronidazole treatment (15.1% [11/73] vs 8.2% [6/73]; $P = 0.2$). Furthermore, for those infants who received broad-spectrum antibiotic in combination with metronidazole, we further compared the administration time of metronidazole between stage II group (n = 62) and stage III group (n = 11), and found no significant difference (10.82 ± 4.84 vs 10.18 ± 3.28 days; $P = 0.68$). This result suggested that the administration time of metronidazole did not affect the process of severity of NEC. For those infants with stage III NEC (n = 17), we also found the mortality of infants who received metronidazole was not lower than that who did not receive metronidazole (9.1% vs 33.3%; $P = 0.56$). Thus, the above results suggested that broad-spectrum antibiotic plus metronidazole may not prevent NEC deteriorating from stage II to III in full-term and near-term infants.

The Risk Factors Associated With Severity of NEC

On the basis of the above finding, an interesting question was put forward on which factor was associated with the process of stage II deteriorating to stage III. In order to find those risk factors, we used the PS-matched NEC cases to build a case-control study. None of the maternal factors such as prolonged rupture of membranes (>18 hours), and the demographics such as gender, were significantly associated with NEC progression (Table 4). The period of using broad-spectrum antibiotics in the 2 groups were similar (an average of 14 days; $P = 0.14$). An initial bivariate analysis showed that many factors were associated with deterioration of NEC (Fig. 1). In general, sepsis after NEC, transfusion of blood products (cryoprecipitation, plasma, and platelet), and the longer time for nasogastric suction and cessation of enteral feeding after diagnosis of NEC were significantly associated with the deterioration of definite NEC (Table 5).

These statistically significant indicators were tested again by binary logistic regression analysis, and we found that patients needed to use blood products (cryoprecipitation, plasma) after diagnosis of NEC (odds ratio [OR] 8.003, 95% confidence interval [CI] 2.365–27.087, $P = 0.00$), showed sepsis after NEC (OR 3.748, 95% CI 1.171–11.998, $P = 0.03$), and needed longer time for nasogastric suction; these were the risk factors for stage II NEC progressing to stage III (OR 1.102, 95% CI 1.004–1.21, $P = 0.04$). We further analyzed the pathogen of sepsis, and found 6 strains of bacteria as follows: *Eagglomerans* (1 strain), *Staphylococcus epidermidis* (2 strains), and *Klebsiella pneumonia* (3 strains).

DISCUSSION

Many factors were involved in the pathogenesis of NEC.[20,21] Although abnormal microbial intestinal colonization and a reduction in diversity of gut microbiome played an important role in the pathogenesis of NEC, no single microorganism has been identified.[20] Because anaerobic bacteria existed in the

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TABLE 2. The Complications of NEC Infants Treated by Broad-spectrum Antibiotics Plus (With/Without) Metronidazole

		Before PS-matching (% [n])			After PS-matching (% [n])		
	Total feature	Without (n = 74)	With (n = 155)	*P*	Without (n = 73)	With (n = 73)	*P*
Metabolic acidosis, % (n)	4.4 (10)	2.7 (2)	5.2 (8)	0.61	2.7 (2)	4.1 (3)	1
Intracranial hemorrhage, % (n)	8.3 (19)	9.5 (7)	7.7 (12)	0.66	9.6 (7)	4.1 (3)	0.19
Scleredema neonatorum, % (n)	5.7 (13)	4.1 (3)	6.5 (10)	0.67	4.1 (3)	4.1 (3)	1
Hypoglycemia, % (n)	4.8 (11)	5.4 (4)	4.5 (7)	1	5.5 (4)	4.1 (3)	1
Hyperglycemia, % (n)	3.9 (9)	1.4 (1)	5.2 (8)	0.31	1.4 (1)	2.7 (2)	1
Coagulopathy, % (n)	33.2 (76)	45.9 (34)	27.1 (42)	0.01	45.2 (33)	39.7 (29)	0.5
Ventricular septal defect, % (n)	3.9 (9)	5.4 (4)	3.2 (5)	0.67	5.5 (4)	2.7 (2)	0.68
Atrial septal defect, % (n)	33.6 (77)	40.5 (30)	30.3 (47)	0.13	39.7 (29)	34.2 (25)	0.49
Patent ductus arteriosus, % (n)	8.3 (19)	5.4 (4)	9.7 (15)	0.27	5.5 (4)	8.2 (6)	0.51
Sepsis, % (n)	18.8 (43)	20.3 (15)	18.1 (28)	0.69	20.5 (15)	20.5 (15)	1
Hemolytic disease of newborn, % (n)	15.3 (35)	13.5 (10)	16.1 (25)	0.61	13.7 (10)	17.8 (13)	0.5
Hyperlactacidemia,% (n)	5.7 (13)	4.1 (3)	6.5 (10)	0.67	4.1 (3)	5.5 (4)	1
Hypernatremia, % (n)	1.7 (4)	1.4 (1)	1.9 (3)	1	1.4 (1)	2.7 (2)	1
Hyponatremia, % (n)	6.6 (15)	5.4 (4)	7.1 (11)	0.84	5.5 (4)	5.5 (4)	1
Hyperkalemia, % (n)	4.4 (10)	6.8 (5)	3.2 (5)	0.38	6.8 (5)	2.7 (2)	0.44
Hypokalemia, % (n)	3.9 (9)	3.9 (6)	4.1 (3)	1	4.1 (3)	4.1 (3)	1
Hyperchloremia, % (n)	2.2 (5)	2.7 (2)	1.9 (3)	1	2.7 (2)	1.4 (1)	1
Hypochloremia, % (n)	4.8 (11)	4.1 (3)	5.2 (8)	0.97	4.1 (3)	2.7 (2)	1
Hypocalcemia, % (n)	9.6 (22)	9.5 (7)	9.7 (15)	0.96	9.6 (7)	5.5 (4)	0.35
Hepatic dysfunction, % (n)	1.7 (4)	2.7 (2)	1.3 (2)	0.82	2.7 (2)	0	0.48
Renal dysfunction, % (n)	8.7 (20)	10.8 (8)	7.7 (12)	0.442	11 (8)	6.8 (5)	0.38
Cholestasis, % (n)	1.7 (4)	1.4 (1)	1.9 (3)	1	1.4 (1)	0	1

NEC = necrotizing enterocolitis, PS = propensity score.

intestinal flora[8] and antianaerobic regimen produced a significant reduction in early mortality of experimental intra-abdominal sepsis,[22] the empirical inclusion of antianaerobic antimicrobial agents was recommended for NEC infants.[14] However, Faix et al[8] found no difference in mortality in those who received broad-spectrum antibiotics plus antianaerobic regimen compared with those who only received broad-spectrum antibiotics therapy in a small cohort study. Autmizguine et al[7] further found that the anaerobic antimicrobial therapy did not prevent the severity of NEC.

TABLE 3. The Treatment Protocols and the Laboratory Test (Within 24 h of NEC Diagnosis) of NEC Infants Treated by Broad-spectrum Antibiotics Plus (With/Without) Metronidazole

		Before PS-matching (% [n])			After PS-matching (% [n])		
Variables	Total Feature	Without (n = 74)	With (n = 155)	*P*	Without (n = 73)	With (n = 73)	*P*
WBC $<5\times10^9$/L or $>20\times10^9$/L, % (n)	16 (37)	21.6 (16)	13.5 (21)	0.12	21.9 (16)	15.1 (11)	0.29
Platelet $<100\times10^9$/L, % (n)	8.3 (19)	9.5 (7)	7.7 (12)	0.66	9.6 (7)	8.2 (6)	0.77
C-reaction protein >8mg/L, % (n)	31 (71)	24.3 (18)	34.2 (53)	0.13	24.7 (18)	35.6 (26)	0.15
Days for first cessation of enteral feeding, IQR, d	0 (0–1)	0 (0–1)	0 (0–1)	0.49	0 (0–1)	0 (0–1)	0.78
Days for first nasogastric suction, IQR, d	0 (0–0)	0 (0–1)	0 (0–0)	0.82	0 (0–0)	0 (0–0)	0.59
Days for broad spectrum antibiotics, IQR, d	0 (0–1)	0 (0–1.25)	0 (0–1)	0.97	0 (0–1.5)	0 (0–2)	0.96
Probiotics, % (n)	9.2 (21)	16.2 (12)	12.3 (19)	0.41	16.4 (12)	13.7 (10)	0.64
Dopamine support, % (n)	2.2 (5)	2.7 (2)	1.9 (3)	1	2.7 (2)	1.4 (1)	1
Aminophylline, % (n)	1.3 (3)	0	1.9 (3)	0.55	0	4.1 (3)	0.24
Intravenous immunoglobulin, % (n)	3.1 (7)	2.7 (2)	3.2 (5)	1	2.7 (2)	2.7 (2)	1
Transfusion, % (n)	6.6 (15)	2.7 (2)	8.4 (13)	0.18	2.7 (2)	8.2 (6)	0.28
Albumin, % (n)	9.6 (22)	10. (8)	9 (14)	0.67	11 (8)	8.2 (6)	0.57

IQR = interquartile range, NEC = necrotizing enterocolitis, PS = propensity score, WBC = white blood cell.

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TABLE 4. Comparison of Features Between Stage II and III NEC Infants

	Stage II (n = 129)	Stage III (n = 17)	*P*
PIH, % (n)	4.7 (6)	0	1
ICP, % (n)	0.8 (1)	0	1
Vaginal delivery, % (n)	40.3 (52)	41.2 (7)	0.95
PROM, % (n)	4.7 (6)	0	1
Amniotic fluid contamination, % (n)	10.1 (13)	0	0.36
Infants of diabetic mother, % (n)	3.1 (4)	0	1
Gestational age, IQR, weeks	38.86 (37.43–39.71)	39 (36.93–39.57)	0.72
Birth weight (± SD), g	2913.81 ± 505.78	2879.41 ± 443.04	0.79
Gender (male), % (n)	57.4 (74)	58.8 (10)	0.91
Multiple gestations, % (n)	4.7 (6)	11.8 (2)	0.24
Breast fed at home, % (n)	21.7 (28)	23.5 (4)	0.51
The age of onset, IQR, d	7 (1–12)	6 (1–10)	0.98
The age of diagnosis, IQR, d	9.25 (3–17)	7 (3–21)	0.75

ICP = intrahepatic cholestasis of pregnancy, IQR = interquartile range, NEC = necrotizing enterocolitis, PIH = pregnancy-induced hypertension, PROM = prolonged rupture of membranes >18 h.

Studies had shown that T-helper 17 (Th17) cells, subtypes of T lymphocytes, can produce interleukin (IL)-17, and have close relationship with the development of mucosal immunity and intestinal diseases.[23,24] IL-17 is a proinflammation factor, and it can lead to the impairment of intestine such as inflammatory bowel disease.[24] However, it also has protective effects in immunity to bacterial and fungal pathogens.[25] Anaerobic bacterium played a complex role in the development of Th17 cells.[26,27] Segmented filamentous bacteria, one of the anaerobic bacterium, have been proven to be a specific inducer of Th17.[26] Whereas other anaerobic bacteria such as *Bacteroides fragilis*, *Lactobacilli*, and *Bifidobacteria* can repress the differentiation of Th17 cells.[27,28] Thus, it is difficult to judge the influence of metronidazole on the production of IL-17 and the latter's anti-inflammation or proinflammation role because administration of metronidazole may either promote the induction or repress the differentiation of the Th17 cells.

We found that anaerobic antimicrobial therapy did not reduce the mortality of infants who deteriorated to stage III perhaps due to a small sample size (n = 17) in our study. For preterm infants with birth weight less than 1500 g, Autmizguine et al[7] further found that broad-spectrum antibiotic plus anaerobic antimicrobial strategies reduced the mortality of infants with surgical NEC, in comparison with only using broad-spectrum antibiotic therapy. However, the reduced mortality for surgical NEC may not just contribute to the effects of anaerobic antimicrobial therapy because the abdominal surgery converted the gut ecosystem from an anaerobic to an aerobic system,[29] which was not suitable for the growth of anaerobic bacteria. Hence, the reduced mortality for surgical NEC perhaps needs further study. In the present study, we found the rate for deterioration of NEC from stage II to III had no significant difference between the group with metronidazole treatment and the group without metronidazole treatment ($P = 0.2$), and in infants with stage III NEC, and the mortality in the group with metronidazole treatment and the group without metronidazole treatment was similar. Our result might suggest that broad-spectrum antibiotics plus metronidazole therapy may also not prevent the deterioration from stage II to III in full-term and near-term infants. Above all, these data

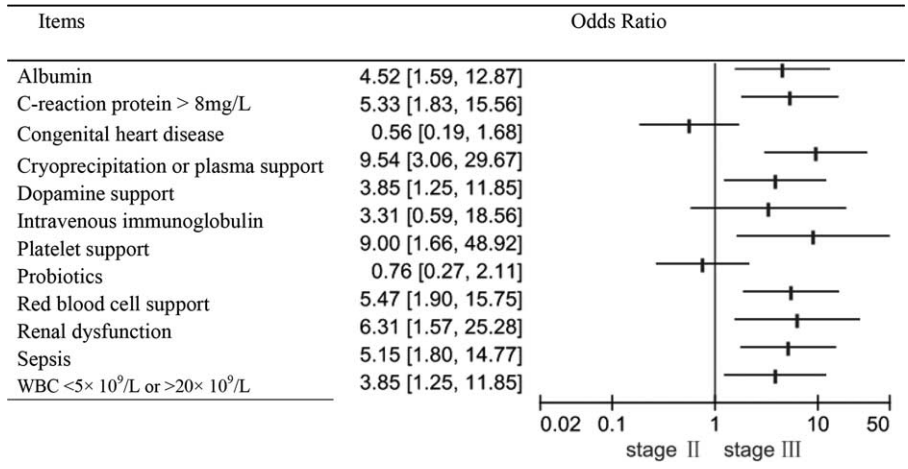

FIGURE 1. The risk factors associated with the deterioration of necrotizing enterocolitis from stage II to III.

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TABLE 5. Comparison of Complications, Treatment Protocols and Laboratory Test (Within 24 h of NEC Diagnosis) Between Stage II and III NEC Infants

	Stage II (n = 129)	Stage III (n = 17)	*P*
Sepsis, % (n)	21.7 (28)	58.8 (10)	0.00
Renal dysfunction,% (n)	4.7 (6)	23.5 (4)	0.02
Congenital heart disease, % (n)	42.6 (55)	29.4 (5)	0.3
Hemolytic disease of newborn, % (n)	15.5 (20)	17.6 (3)	1
Asphyxia, % (n)	3.1 (4)	0	1
Intracranial hemorrhage, % (n)	7.8 (10)	0	0.5
Scleredema neonatorum, % (n)	3.1 (4)	11.8 (2)	0.15
Platelet $<100 \times 10^9$/L, % (n)*	7.8 (10)	17.6 (3)	0.37
WBC $<5 \times 10^9$/L or $>20 \times 10^9$/L, % (n)*	12.4 (16)	35.3 (6)	0.03
C-reaction protein >8 mg/L, % (n)*	25.6 (33)	64.7 (11)	0.00
Need time for cessation of enteral feeding after diagnosis of NEC, IQR, d	7 (4–9)	9 (6–11.5)	0.02
Need time for nasogastric suction after diagnosis of NEC, IQR, d	0 (0–4.5)	6 (2–8)	0
Days for broad spectrum antibiotics after diagnosis of NEC, IQR, d	13 (10–17)	15 (10–26)	0.14
Dopamine support, % (n)	12.4 (16)	35.3 (6)	0.03
Probiotics, % (n)	48.1 (62)	41.2 (7)	0.59
Red blood cell support, % (n)	17.1 (22)	52.9 (9)	0.00
Platelet support, % (n)	2.3 (3)	17.6 (3)	0.02
Cryoprecipitation or plasma support, % (n)	8.5 (11)	47.1 (8)	0
Albumin, % (n)	24 (31)	58.8 (10)	0.01
Intravenous immunoglobulin, % (n)	3.9 (5)	11.8 (2)	0.19

IQR = interquartile range, NEC = necrotizing enterocolitis, WBC = white blood cell.
* Data were obtained within 24 h after NEC was diagnosed.

point to the importance of further research to find out the optimal antibiotic therapy in infants with NEC.

The association between transfusions and the development of NEC had been identified in some studies,[30–32] and transfusion of packed red blood cells was also associated with the severity of NEC.[3] Infants with NEC always had several hematological abnormalities such as thrombocytopenia and coagulopathy.[33,34] Thus, platelet, fresh frozen plasma, and/or cryoprecipitate were often used to support those infants. We further analyzed the association between these support managements and the severity of NEC by logistic regression analysis, and found that transfusion of these blood products after NEC diagnosis was an independent risk factor for deterioration of NEC. Therefore, those infants with stage II NEC needing fresh frozen plasma and/or cryoprecipitate supports might be more likely to deteriorate to stage III.

We found that sepsis after NEC was an independent factor for deterioration of NEC, and this result was consistent with previous studies.[3,35] Approximately 20% to 30% of infants with NEC subsequently developed sepsis.[36] Due to bacterial translocation after damage of intestinal epithelial cell after NEC, infants with NEC were prone to develop sepsis.[37] In turn, sepsis could induce further necrosis of intestinal epithelial cells.[38,39] Therefore, vicious cycle might be established between NEC and sepsis. Meanwhile, antibiotics regimen for sepsis was based on empiric therapy because of low positive rate of blood culture in infants with sepsis.[40] Therefore, it is hard to block this vicious cycle which easily leads to the deterioration of NEC. Without any doubt, determining the microorganism in sepsis after NEC will be helpful for targeted antimicrobial therapy which might break the vicious cycle. However, it was quite difficult to identify the profile of pathogens after NEC in our study because only 6 strains were identified in positive blood cultures.

There are still some limitations in our study, including the inherent errors and bias of retrospective studies. This study was also limited by its cohort design and we could not completely avoid the risk of unobserved confounders which might affect the PS. The formula used for all infants during hospitalization is another limitation of this study. Studies had shown that breast milk could decrease the incidence of NEC,[21,41,42] and lack of early colostrums feeding would contribute to the deterioration of NEC.[3] We did not know whether formula feeding could have an influence on the efficiency of broad-spectrum antibiotic plus metronidazole therapy. Moreover, we noticed that the mortality in metronidazole group was lower than that in the group without metronidazole treatment. Similarly, for those infants with stage III NEC, the mortality was lower in the metronidazole-treated group than in the group without metronidazole treatment. These differences are clinically significant. Although the mortality between the group with metronidazole treatment and the group without metronidazole treatment exhibited no statistical difference, they might be due to in part the small sample size in our study. Therefore, these limitations could be addressed through further prospective study.

In conclusion, we found that broad-spectrum antibiotic plus metronidazole may not prevent the deterioration of NEC in full-term and near-term infants in the present study. Those infants who had sepsis and required transfusion of blood product supports after stage II NEC were more likely to progress to stage III. Whether broad-spectrum antibiotic plus metronidazole could improve the survival rate of those infants with stage III NEC need further study.

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