Association of anemia and platelet activation with necrotizing enterocolitis with or without sepsis among low birth weight neonates: a case-control study

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Abstract

Background To evaluate the value of the proportion of large platelets (PLCR) and platelet crit (PCT) in predicting necrotizing enterocolitis (NEC) in low birth weight (LBW) neonates.

Methods A total of 155 LBW (< 2500 g) neonates with NEC admitted to the neonatal intensive care unit (NICU) of the hospital from January 1, 2017 to November 30, 2019 were included in the case group. According to the 1:3 case-control study design, a total of 465 LBW neonates without NEC (3 for each LBW neonate with NEC) in the NICU born ≤ 24 h before or after the birth of the cases were included in the control group.

Results During the study period, a total of 6946 LBW neonates were born, and 155 had NEC, including 98 who also had sepsis. Neonatal sepsis was the most important risk factor and confounding factor for NEC in LBW neonates. Further stratified analysis of the sepsis showed that in LBW neonates without sepsis, anemia, high PLCR, and high PCT increases the risk of NEC, and the receiver operating characteristic curve area of PLCR was 0.739, the sensitivity was 0.770, the specificity was 0.610, and the cutoff value was 33.55.

Conclusions: The data showed that 2/100 LBW neonates were prone to NEC, and stratified analysis of confounding factors of sepsis identified the risk factors of NEC in LBW neonates. This study first reported the significant value of PLCR in the early prediction of NEC occurrence in LBW neonates without sepsis.

What Is Known

- Neonatal necrotizing enterocolitis (NEC) is common among premature infants, severely threatening the life of neonates.
- Lots of risk factors have been declared to trigger NEC, such as blood transfusion, anemia and neonatal sepsis., but no consensus has been reached.
- The onset of NEC is closely related to sepsis, but sepsis has a strong heterogeneity, which may confuse the diagnosis and cognition of clinical diseases.

What Is New

- Sepsis is an important confounding factor of NEC and needs to be analyzed separately.
- The risk factors of NEC patients with sepsis and without sepsis are different.
- A significant value of PLCR, rather than PLT count, in the early prediction of NEC occurrence in LBW neonates without sepsis.

Background

Neonatal necrotizing enterocolitis (NEC) is a neonate-specific inflammatory necrotizing disease that involves ileum and/or colon and is common among premature infants, severely threatening the life of neonates¹⁻³. With continuous advances in the treatment of premature infants in recent years, the mortality rate has declined, but the incidence of NEC has been rising sharply. Statistically, the incidence of NEC in premature infants of birth weight < 1500 g was 5–10%, and the mortality was 20–30%, and > 30–50% of NEC neonates need surgical treatment^{4,5}. Despite decades of research, the current understanding of the diagnosis and treatment of neonatal NEC is limited, the mortality of neonatal NEC remains high, and the advances in neonatal surgery have not significantly improved the prognosis in NEC survivors^{6,7}. Therefore, intervening in NEC, especially NEC in LBW neonates, should be under intensive focus with respect to the identification of causes and related factors for early diagnosis and treatment.

Hitherto, the pathogenesis of NEC is unclear, but several studies have shown that it is caused by a combination of factors. Some studies reported that preterm birth^{6,7}, low birth weight⁶⁻⁹, and race⁸ were critical risk factors. Recent studies have shown that maternal infection¹⁰, congenital pneumonia¹¹, asphyxia¹², blood transfusion¹², anemia^{13,14}, and neonatal sepsis are also potential contributing factors. Furthermore, NEC pathogenesis has been suggested to be multifactorial, involving a combination of abnormal bacterial colonization, gut prematurity, and ischemia-reperfusion (I/R) injury. NEC caused by the latter two pathologies is not significantly associated with sepsis. Based on the epidemiological and clinical theories, sepsis can confound the diagnosis of the clinical complications and the use of inflammatory proteins as the marker for NEC. Both sepsis and NEC require careful differential diagnosis, as both may be lethal if not diagnosed and treated appropriately.

Regarding gut prematurity, the degree and duration of thrombocytopenia in these infants are associated with the severity of bowel injury and adverse clinical outcomes. Infants with a confirmed diagnosis of NEC develop thrombocytopenia with platelet (PLT) count < 100 × 10⁹/L, this low PLT counts is yet an unresolved clinical dilemma^{15,16}. A robust NEC biomarker different from that of sepsis could improve bedside management, reduce morbidity and mortality, and allow patients to select potential treatments in the clinic. Animal studies¹⁷ showed that PLT activation during NEC-like intestinal injury is an early, thrombin-mediated process that antedates both mucosal damage and the rise in bacterial products in plasma, and hence, is a crucial pathophysiological event during neonatal intestinal injury. In clinical practice, the PLT count in NEC patients is monitored periodically, but PLT activation indicators, such as the mean platelet volume (MPV), platelet crit (PCT), platelet distribution width (PDW), and platelet large cell ratio (PLCR), are usually neglected^{18,19}. Such PLT indicators have valuable in clinical diagnosis and prognosis prediction of cardiovascular and metabolic diseases^{20,21}; however, their clinical significance, reference value, and utilization value are still being investigated.

Intriguingly, the high mortality rate of NEC patients could be ascribed to the difficulty in diagnosing and treating the condition in a timely manner. Radiographic evidence, such as pneumatosis intestinalis, is used to diagnose severe or advanced disease but has a sensitivity of only 44% with limited specificity and lacking concordance in interpretation^{2,22}. Several studies have adopted 1:1 or 1:2 case-control

cohorts with low statistical efficiency^{23,24}. Herein, we hypothesized that sepsis, anemia, and PLT activation index are vital predictors of low body weight NEC (LBW NEC) among all the factors. Furthermore, a 1:3 case-control study with sufficient statistical efficiency was conducted to verify the predictive value of anemia, sepsis, and PLT activation indexes in the incidence of LBW NEC, in order to achieve early diagnosis and treatment and reduce sequelae and mortality.

Methods

Participants

A total of 155 LBW (< 2500 g) neonates with NEC born in the Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China and admitted to the neonatal intensive care unit (NICU) of the hospital from January 1, 2017 to November 30, 2019 were included in the case group. According to the 1:3 case-control study design, a total of 465 LBW neonates without NEC (3 for each LBW neonate with NEC) in the NICU born ≤ 24 h before or after the birth of the cases were included in the control group.

The exclusion criteria for cases and controls were as follows: Neonates were excluded if they were unlikely to survive or had significant gastrointestinal anomalies or if they were discharged at their own will in three days.

Neonates were diagnosed with NEC (stage II or above) if they met the diagnostic standard in *Practical Neonatology* and Bell staging^{25,26}, had clinical symptoms of abdominal distension, vomiting, and bloody stool triad; also, the abdominal plain X-ray scan revealed abdominal intestinal aeration, intestinal obstruction, or intestinal pneumatosis. Together, these findings suggested NEC with definite intestinal pneumatosis and intrahepatic portal venous gas.

Identification of factors

The main influencing factors of the included subjects were listed, and clinical information, including maternal factors as age, nationality, number of fetuses, hypertensive disorders complicating pregnancy (HDCP), gestational diabetes mellitus (GDM), placenta previa (PP), placenta abruption, and premature rupture of membrane (PROM) and neonatal factors, such as date of birth, birth weight, NEC diagnosis age (the time span from the date of birth to diagnosis of NEC), neonatal sepsis, patent ductus arteriosus (PDA), anemia, hypoglycemia, birth asphyxia, blood transfusion, mycoplasma infection, and hyperglycemia, of the subjects were obtained from the medical record system of the hospital every three months. The complications in the included subjects occurred before the diagnosis of NEC. PLT indicators (including PTL count, PDW, MPV, PCT, and PLCR) of the subjects were recorded as the latest data two days before the date of NEC diagnosis, while those of the controls were recorded one day before and after the date of NEC diagnosis of the subjects.

Assignment of main study variables

Dependent variables

Neonates with NEC were assigned 1, and the controls were assigned 0.

Independent variables

Independent variables were qualitative data, and values were assigned values. Since there were no definitive clinical reference values for PLT indicators for neonates, especially for neonates with LBW or low gestational age, the independent variables were stratified and assigned values using the quartile (Q) method, namely quartile 1 (Q1), quartile 2 (Q2), and quartile 3 (Q3) (Table 1).

Table 1

Assignments of independent variables

Variable		Assignment	Variable		Assignment
Maternal factor			Neonatal factor		
Gestational weeks	≤28	0	Gender	Male	0
	29–37	1		Female	1
	≥37	2	Birth weight (g)	<1000	0
Number of fetuses	Single fetus	0		1000–1500	1
	Multiple fetuses	1		1500–2500	2
HDCP	No	0	Transfusion	No	0
	Yes	1		Yes	1
GDM	No	0	Sepsis	No	0
	Yes	1		EOS	1
				LOS	2
PP	No	0	PDA	No	0
	Yes	1		Yes	1
Placenta abruption	No	0	Anemia	No	0
	Yes	1		Yes	1
PROM	No	0	Hypoglycemia	No	0
	Yes	1		Yes	1
			Asphyxia	No	0
				Yes	1
			Mycoplasma infection	No	0
				Yes	1
			Hyperglycemia	No	0
				Yes	1
			PLT	<Q1: 0; Q1-Q2: 1; Q2-Q3: 2; ≥Q3: 3;	
			PDW	<Q1: 0; Q1-Q2: 1; Q2-Q3: 2; ≥Q3: 3;	
			MPV	<Q1: 0; Q1-Q2: 1; Q2-Q3: 2;	

		≥Q3: 3;
	PCT	<Q1: 0; Q1-Q2: 1; Q2-Q3: 2; ≥Q3: 3;
	PLCR	<Q1: 0; Q1-Q2: 1; Q2-Q3: 2; ≥Q3: 3;

Statistical analysis

The general characteristic information was compared between the case and control groups. T-test was used for between-group comparison of quantitative data. Spearman's method was used for the analysis of gestational week, birth weight, and NEC diagnosis age. In the 1:3 case-control analysis, the occurrence of NEC was evaluated by analyzing the variables using univariate non-conditional logistic regression. A factor with $P < 0.5$ was analyzed using stepwise multivariate conditional logistic regression (Cox model). The factors, after stratified by sepsis, were analyzed using univariate and multivariate non-conditional logistic regression. The receiver operating characteristic (ROC) curve was used to evaluate the value of PLCR and PCT in NEC diagnosis. SPSS 22.0 software was used for statistical analyses and image drawing. Data conforming to the normal distribution were expressed as mean $\pm$ standard deviations, while those not conforming to the normal distribution were expressed as median (minimum, maximum). The odds ratio (OR) and the 95% confidence interval (CI) were calculated. An OR with 95% CI not containing 1 was of statistical significance. All tests were two sided, and $P < 0.05$ was considered as statistically significant.

Results

Results of general information

During the study period, a total of 58507 mothers gave birth, and a total of 60182 neonates were born in the hospital. Among them, 6946 (11.54%) had LBW, and 155 (2.23%) had LBW and NEC (including 92 (59.35%) who also had sepsis). Among the 465 neonate controls with LBW in this study, 58 (12.47%) also had sepsis. Among the 6946 LBW neonates, the birth weight of 5270 (75.87%) was between 1500 and 2500 g (including 97 (1.84%) with NEC), the birth weight of 1207 (17.38%) was between 1000 and 1500 g (including 47 (3.89%) with NEC), and the birth weight of 469 (6.75%) was < 1000 g (including 11 (2.35%) with NEC), as shown in Figure 1. Moreover, one of the NEC neonates had a Lahu nationality mother, and the other mothers were Han nationality. General information of LBW neonates and their mothers were showed in table 2.

Table 2

General information of LBW neonates and their mothers in the case-control study

Factors		Case group (n=155)	Control group (n=465)
Maternal factor			
Age (years)		31.35±4.59	31.10±4.51
Nationality (n)	Han Chinese	154	465
	Minority	1	0
Gestational week (median (minimum, maximum))		31 (21, 39)	34 (24, 39)
Number of fetuses (n)	Single fetus	104	288
	Multiple fetuses	51	177
HDCP (n)		18	55
GDM (n)		27	72
PP (n)		18	51
Placenta abruption (n)		20	23
PROM (n)		49	131
Neonatal factor			
Gender (n)	Male	86	226
	Female	69	239
Blood transfusion (n)		18	62
Birth weight (g)		1588.32±384.70	1878.93±449.60
Sepsis (n)		92	58
EOS		22	36
LOS		70	22
PDA (n)		34	82
Anemia (n)		21	20
Hypoglycemia (n)		15	51
Asphyxia (n)		22	68
Mycoplasma infection (n)		11	16
Hyperglycemia (n)		11	10
PLT (10 ⁹ /L)		321.73±105.60	310.32±110.62

PDW (fl)	14.93±2.92	13.17±3.02
MPV (fl)	11.77±0.87	11.00±1.07
PCT (%)	0.38±0.11	0.34±0.12
PLCR (%)	37.77±6.60	31.89±8.13

Correlation between birth weight and gestational week and NEC diagnosis age

During the perinatal period, the development and maturity of the immune system of perinatal infants gradually reduce the incidence of related diseases, which is less likely in premature infants, especially those with LBW. Spearman's correlation analysis showed that the NEC diagnosis age was significantly negatively correlated with birth weight ($P < 0.001$, $R^2 = 0.298$) and gestational week of the mother ($P < 0.001$, $R^2 = 0.243$) (Figure 2), while NEC diagnosis age was not correlated with the complicated sepsis, as shown in Figure S1. The analysis also showed that the birth weight of NEC neonates with sepsis was lower than that of NEC neonates without sepsis ($t = 2.658$, $P = 0.009$), and that birth weight was not correlated with anemia ($t = -0.534$, $P = 0.594$) (Figure 3).

Univariate analysis between NEC occurrence and baseline characteristics and clinical information of mother

The cause of NEC is complex and might be affected by multiple maternal factors (such as general characteristics and complications). According to the univariate analysis of the main complications, the gestational week ($P < 0.001$, OR = 0.321, 95% CI: 0.201–0.512) and placenta abruption ($P = 0.001$, OR = 2.847, 95% CI: 1.517–5.343) were statistically correlated, while HDCP ($P = 0.943$, OR = 0.979, 95.0% CI: 0.556–1.725), GDM ($P = 0.569$, OR = 1.151, 95% CI: 0.709–1.870), PP ($P = 0.825$, OR = 1.067, 95% CI: 0.603–1.888), multiple fetuses ($P = 0.249$, OR = 0.798, 95% CI: 0.544–1.171), and PROM ($P = 0.414$, OR = 1.18, 95% CI: 0.800–1.750) were not statistically correlated with NEC occurrence in LBW neonates (Table 3).

Table 3

Univariate analysis between NEC occurrence and baseline characteristics and clinical information of mother

	B	SE	P	OR	95.% CI	
					Lower Limit	Upper Limit
Gestational week	-0.272	0.036	<0.001	0.321	0.201	0.512
HDCP	-0.021	0.289	0.943	0.979	0.556	1.725
GDM	0.141	0.248	0.569	1.151	0.709	1.870
PP	0.064	0.291	0.825	1.067	0.603	1.888
Multiple fetuses	-0.226	0.196	0.249	0.798	0.544	1.171
Placenta abruption	1.046	0.321	0.001	2.847	1.517	5.343
PROM	0.16	0.201	0.414	1.18	0.800	1.750

Univariate analysis between NEC occurrence and baseline characteristics and clinical information of LBW neonates

According to literature, a correlation has been in the spotlight between NEC occurrence and the baseline characteristics and complications in neonates. The main complications were analyzed using univariate regression in this study. The results showed that in neonates, early-onset sepsis (EOS) ($P < 0.001$, OR = 0.051, 95% CI: 0.029–0.088), late-onset sepsis (LOS) ($P < 0.001$, OR = 0.186, 95% CI: 0.090–0.383), PDA ($P = 0.018$, OR = 1.680, 95% CI: 1.094–2.580), anemia ($P < 0.001$, OR = 3.482, 95% CI: 1.835–6.627), hyperglycemia ($P = 0.005$, OR = 3.476, 95% CI: 1.447–8.351), and birth weight ($P = 0.001$, OR = 0.606, 95% CI: 0.453–0.812) were statistically correlated, while hypoglycemia ($P = 0.651$, OR = 0.870, 95% CI: 0.474–1.596), asphyxia ($P = 0.895$, OR = 0.966, 95% CI: 0.575–1.623), wet lung ($P = 0.058$, OR = 1.483, 95% CI: 0.986–2.231), mycoplasma infection ($P = 0.059$, OR = 2.144, 95% CI: 0.973–4.725), and blood transfusion ($P = 0.580$, OR = 0.854, 95% CI: 0.488–2.231) were not statistically correlated with NEC occurrence in LBW neonates. In addition, the analysis of the correlation between NEC occurrence and PLT indicators in LBW neonates showed that the PLT count ($P = 0.167$, OR = 1.494, 95% CI: 0.953–1.322) was not statistically correlated, while PDW ($P < 0.001$, OR = 1.920, 95% CI: 1.597–2.307), MPV ($P < 0.001$, OR = 2.093, 95% CI: 1.735–2.525), PCT ($P < 0.001$, OR = 1.441, 95% CI: 1.217–1.705), and PLCR ($P < 0.001$, OR = 2.156, 95% CI: 1.777–2.017) were statistically correlated with NEC occurrence in LBW neonates (Table 4).

Table 4

Univariate analysis between NEC occurrence and baseline characteristics and clinical information of LBW neonates

	B	SE	P	OR	95% CI	
					Lower Limit	Upper Limit
Sepsis	2.327	0.216	<0.001	10.247	6.717	15.633
EOS	2.977	0.279	<0.001	0.051	0.029	0.088
LOS	1.682	0.368	<0.001	0.186	0.090	0.383
PDA	0.519	0.219	0.018	1.680	1.094	2.580
Anemia	1.249	0.328	<0.001	3.482	1.835	6.627
Hypoglycemia	-0.140	0.310	0.652	0.870	0.474	1.596
Asphyxia	-0.035	0.265	0.895	0.966	0.575	1.623
Wet lung	0.394	0.208	0.058	1.483	0.986	2.231
Mycoplasma infection	0.763	0.403	0.059	2.144	0.973	4.725
Hyperglycemia	1.246	0.447	0.005	3.476	1.447	8.351
Blood transfusion	-0.158	0.285	0.580	0.854	0.488	1.494
Birth weight	-0.500	0.149	0.001	0.606	0.453	0.812
PLT	0.115	0.084	0.167	1.122	0.953	1.322
PDW	0.652	0.094	<0.001	1.920	1.597	2.307
MPV	0.739	0.096	<0.001	2.093	1.735	2.525
PCT	0.365	0.086	<0.001	1.441	1.217	1.705
PLCR	0.768	0.099	<0.001	2.156	1.777	2.017

Analysis of the effect of multiple factors and their correlation on NEC occurrence

In order to avoid missing the critical clinical factors, 16 variables with $P < 0.5$ in the univariate regression analysis result were taken as independent variables for the 1:3 case-control analysis using stepwise multivariate conditional logistic regression (the survival function was assessed by Cox regression). The results showed that sepsis, PLCR, and PCT were the final factors into the regression model. EOS ($P = 0.001$, OR = 2.424, 95% CI: 1.461–4.021), LOS ($P < 0.001$, OR = 4.291, 95% CI: 3.001–6.138), and anemia ($P = 0.030$, OR = 1.675, 95% CI: 1.053–2.665) increased the risk of NEC in LBW neonates, and increased PLCR ($P < 0.001$, OR = 1.451, 95% CI: 1.220–1.724) and increased PCT ($P = 0.007$, OR = 1.225, 95% CI: 1.056–1.422) could be the risk factors for NEC occurrence, as shown in Table 5.

Table 5

Multivariate logistic regression (survival function Cox regression) analysis of NEC occurrence in LBW neonates

	B	SE	P	OR	95% CI	
					Lower Limit	Upper Limit
Sepsis	1.285	0.170	<0.001	3.614	2.589	5.046
EOS	0.885	0.258	0.001	2.424	1.461	4.021
LOS	1.457	0.183	<0.001	4.291	3.001	6.138
Anemia	0.516	0.237	0.030	1.675	1.053	2.665
PLCR	0.372	0.088	<0.001	1.451	1.220	1.724
PCT	0.203	0.076	0.007	1.225	1.056	1.422

Analysis of the effect of multiple factors and their correlation on NEC occurrence in neonates without sepsis

The effect of sepsis weighted the greatest among the above factors of statistical significance. Sepsis leads to systemic inflammatory response, involving multiple organ system damages and altering the evaluation indicators, such as PLT count. [Whereupon](#), the information of NEC patients without sepsis was analyzed using univariate regression (Table S1), and 17 variables with $P < 0.5$ were entered as independent variables for stepwise multivariate non-conditional logistic regression analysis. The results showed that anemia, PLCR, and PCT were the final significant indicators in the model. Anemia ($P = 0.001$, OR = 4.367, 95% CI: 1.853–10.291) increases the risk of NEC in LBW neonates without sepsis, and increased PLCR ($P < 0.001$, OR = 2.222, 95% CI: 1.633–3.023) and PCT ($P = 0.024$, OR = 1.368, 95% CI: 1.042–1.795) could be the indicators to predict the risk of NEC in LBW neonates without sepsis, as shown in Table 6.

Table 6

Multivariate logistic regression analysis of NEC occurrence in LBW neonates without sepsis

	B	SE	P	OR	95% CI	
					Lower Limit	Upper Limit
Anemia	1.474	0.437	0.001	4.367	1.853	10.291
PLCR	0.798	0.157	<0.001	2.222	1.633	3.023
PCT	0.313	0.139	0.024	1.368	1.042	1.795

Analysis of the effect of multiple factors and their correlation on NEC occurrence in neonates with sepsis

The information of NEC patients with sepsis was analyzed using univariate regression (Table S2), and 10 variables with $P < 0.5$ were considered as independent variables for stepwise multivariate non-conditional

logistic regression analysis. The results showed that only MPV was the final significant indicator entered into the model. Increased MPV ($P = 0.040$, OR = 1.409, 95% CI: 1.017–1.953) was the indicator to predict the risk of NEC in LBW neonates with sepsis (Table 7).

Table 7

Multivariate logistic regression analysis of NEC occurrence in LBW neonates with sepsis

	B	SE	P	OR	95% CI	
					Lower Limit	Upper Limit
MPV	0.343	0.167	0.040	1.409	1.017	1.953

Value of PLCR and PCT in predicting NEC occurrence

The ROC curve fitting analysis was used to evaluate the value of PLCR, PCT, and the combination of the two in diagnosing NEC. In all NEC patients in this study, the ROC curve area of PLCR diagnosis was 0.717 ($P < 0.001$), the sensitivity was 0.767, the specificity was 0.581, and the cutoff value was 33.55. The ROC curve area of PCT diagnosis was 0.606 ($P < 0.001$), the sensitivity was 0.640, the specificity was 0.560, and the cutoff value was 0.3350. The ROC curve area of PLCR-PCT diagnosis was 0.719 ($P < 0.001$), the sensitivity was 0.920, the specificity was 0.423, and the cutoff value was 0.1566 (Figure 4).

In NEC patients without sepsis, the ROC curve area of PLCR diagnosis was 0.739 ($P < 0.001$), the sensitivity was 0.770, the specificity was 0.610, and the cutoff value was 33.55. The ROC curve area of PCT diagnosis was 0.629 ($P = 0.001$), the sensitivity was 0.672, the specificity was 0.560, and the cutoff value was 0.3350. The ROC curve area of PLCR-PCT diagnosis was 0.748 ($P < 0.001$), the sensitivity was 0.852, the specificity was 0.557, and the cutoff value was 0.1074. In conclusion, the value of the combination of PLCR and PCT and PCT alone in diagnosing NEC was not significantly higher than PLCR alone (Figure 5).

Discussion

The present study aimed to provide a pooled estimation of NEC in LBW neonates in China. The results showed that 2/100 LBW neonates developed NEC, and sepsis and anemia were the risk factors for NEC occurrence in LBW neonates. This might be the first study to show the superior value of PLT activation (especially PLCR), rather than PLT count, in predicting NEC occurrence in LBW neonates. Most studies postulate that neonatal sepsis is a major risk factor for NEC^{10, 12, 26, 27}. Currently, the correlation between anemia and NEC occurrence is under intensive focus, but the conclusions of such studies are yet controversial. In the study, our results are suggestive that there is a correlation between anemia and NEC occurrence. The present study identified the risk factor for NEC occurrence in LBW neonates in NICUs in China and emphasized the value of PLT activation in diagnosing NEC in LBW neonates, thereby providing a new approach for future studies on the pathogenesis of NEC.

Correlation between neonatal sepsis and NEC occurrence

Stratified analysis of sepsis identified the risk factors for NEC in LBW neonates. In this study, every 6/10 NEC patients presented sepsis, and the NEC diagnosis age was not correlated to sepsis. Both EOS and LOS were risk factors for NEC, suggesting that the control of intrauterine infection and hospital infection is crucial to prevent NEC. In the event of sepsis infection, toxins generated by pathogens may directly damage the intestinal mucosa or activate immune cells to produce cytokines, thereby altering vascular permeability and tissue damage³. These phenomena result in the accumulation of PLTs and white blood cells in capillaries, which in turn, causes intestinal damage and eventually NEC by blocking blood flow, aggravating the intestinal mucosa, and initiating excessive multiplication of intestinal bacteria. Another study showed that sepsis increases the risk of NEC by three-fold²⁶, which is similar to the current conclusion. Typically, neonatal sepsis caused by different types of microbes is manifested by varied pathophysiology and can give rise to several complications and outcomes^{27,28}. Therefore, neonatal sepsis is deemed as a vital confounding factor in the etiological analysis of NEC. Importantly, we also that the risk factors for NEC differed between neonates with and without. These patients were further grouped into NEC subjects with and without sepsis for analysis. The results showed that NEC occurrence was correlated with anemia, PLCR, and PCT in subjects without sepsis under the interaction between multiple factors. However, in NEC subjects with sepsis, only MPV was weakly correlated with NEC occurrence.

Correlation between PLT activation indicators and NEC occurrence

PLT activation, rather than PLT count, was the more early predictor for NEC occurrence. Consistent with the clinical observations in human NEC^{29,30}, pups with TNBS-mediated acute necrotizing ileocolitis showed increased immature PLT fractions, high MPV, and increased megakaryocyte number/ploidy in the bone marrow; these manifestations favor peripheral PLT consumption but not decreased production, as the kinetic basis for thrombocytopenia³¹. However, according to the literature, the PLT count decreased significantly in NEC patients compared to that in non-NEC patients and was correlated with the severity of NEC³². Our results were different from those of the present study. The main reasons may be that the PLT in this study was counted 2 days before NEC diagnosis when NEC is in the early stage and the bone marrow is producing compensatory blood vigorously to maintain normal PLT count or increase PLT count; additionally, PLCR and PCT are sensitive and show an increase at the early stage. Apparently, PLCR and PCT, not PLT count, could be used as indicators to predict the early risk in NEC patients without sepsis, indicating that PLT activation was the early predictor for NEC occurrence. Various types of sepsis affect the PLT through different pathways. PLT activation and depletion occurring during NEC disrupts the mucosal wall established and is secondary to bacterial translocation across the damaged mucosa¹⁷. Damage to vascular endothelium caused by bacteria is repaired by PLTs, resulting in increased platelet consumption, activated immune system, and promoted PLT apoptosis³³. Other common viral infections, such as Torch or fungal infection, directly destroy the megakaryocytes or PLTs, inhibit bone marrow hematopoiesis, induce the production of autoantibodies to accelerate PLT destruction and affect their

levels in peripheral blood. Severe bacterial infections (such as GNB infection) disrupt bone marrow hematopoiesis, leading to thrombocytopenia. Therefore, it could be speculated that the value of PLT indicators in predicting the occurrence of NEC in neonates with sepsis was not significant.

Correlation between anemia and NEC occurrence

Anemia was a risk factor for NEC occurrence in patients without sepsis, but it was not in patients with sepsis. The results of previous studies on anemia promoting NEC occurrence are greatly varied. Patel et al.¹³ and Singh et al.²⁵ speculated that anemia, rather than blood transfusion, was correlated with a high risk of NEC occurrence; however, other retrospective studies did not find such a significant effect^{26,34}. Nonetheless, these studies had not conducted a stratified analysis of sepsis. Herein, we proposed that anemia, rather than blood transfusion, increased the risk of NEC occurrence in patients without sepsis because anemia damages splanchnic perfusion and causes hypoxia, anaerobic metabolism, and accumulation of anaerobic metabolism products, such as lactic acids. These by-products disrupt the intestinal vascular regulation and result in ischemic injury, thereby increasing the risk of NEC occurrence³⁵. Various microbes causing sepsis give rise to anemia, hypoxia, and intestinal tissue damage in the body, and hence, are deemed as confounding factors that complicate the analysis of the results.

Correlation between gestational week and birth weight and NEC occurrence

LBW results in longer NEC occurrence. Some studies showed that LBW, especially extreme LBW, is the risk factor for NEC occurrence^{8,10,11,13}. Wang et al.²⁶ studied the risk factors for NEC occurrence and found that the gestational week and birth weight is correlated with NEC occurrence in univariate analysis, but not in a multivariate logistic analysis. Furthermore, our present study showed that the incidence of NEC varied in neonates with different birth weights and peaked in those of the median birth weight (1000–1500 g) compared to those of extremely low birth weight. However, the multivariate analysis did not show any such correlation, which could be attributed to the following reasons: (1) This study focused only on LBW neonates, and low survival rate of extreme LBW neonates might affect the result; (2) The correlation between sepsis and birth weight caused biases in the univariate analysis results. Interestingly, the birth weight was negatively correlated with the NEC diagnosis age, which was similar to that of the data on gestational week³⁶. This reminds neonatologists to focus on the possibility of NEC occurrence over a prolonged period in neonates of low birth weight, which is conducive to timely diagnosis and accurate treatment of NEC.

Nevertheless, the present study had some limitations. First, all the results were based on the largest women's hospital in Zhejiang Province (it is also one of the largest women's hospitals in China), but the information recorded did not reflect NEC occurrence and the associated risk factors nationwide. Therefore, multicenter clinical studies are essential to further investigate the correlation between anemia, increased PLCR, increased PCT, and NEC occurrence in LBW neonates. Second, this case-control study was retrospective design, and thus, subject to information bias. Hence, a cohort study, as well as a

randomized controlled clinical experiment, should be carried out in the future to substantiate the current findings.

Conclusions

PLCR has significant value in early prediction of the incidence of NEC in LBW neonates without sepsis.

Abbreviations And Acronyms

NEC, necrotizing enterocolitis

LBW, low birth weight

PLCR, the value of the proportion of large platelets

PCT, platelet crit

MPV, mean platelet volume

PDW, platelet distribution width

HDGP, hypertensive disorders complicating pregnancy

GDM, gestational diabetes mellitus

PP, placenta previa

PROM, premature rupture of membrane

PDA, patent ductus arteriosus

Declarations

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Authors' contributions: ZJ designed the study, acquired funding, and was the principal author of the manuscript. GYY supervised data collection, did the analysis and reviewed the manuscript. SYZ

supervised and contributed to the design, supervised the data analysis and co-wrote the manuscript. LZ and obtained epidemiology data and did the analysis.

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Figures

Figure 1

Diagnoses of enrolled infants in 1:3 case-control study

Figure 2

Correlation between NEC diagnosis age and birth weight and gestational week of mother

Figure 3

Correlation between birth weight and anemia and sepsis

Figure 4

Value of PLCR and PCT in diagnosing NEC in LBW neonates

Figure 5

Value of PLCR and PCT in diagnosing NEC in LBW neonates without sepsis

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