Combination of CRP, PCT, N%, Bell stage and add milk the day before onset of NEC for predicting surgical necrotizing enterocolitis and their clinical significance

Li Wang

First Affiliated Hospital of Anhui Medical University

Jingwei Huang

first affiliated hospital of Anhui Medical University

Lili Wang (✉ wllwywz@163.com)

First Affiliated Hospital of Anhui Medical University

Research article

Keywords: neonate, necrotizing enterocolitis, surgery, risk factors, C-reactive protein

Posted Date: November 22nd, 2019

DOI: <https://doi.org/10.21203/rs.2.17689/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Objectives Necrotizing enterocolitis (NEC) is a severe neonatal disease. The present study aimed to identify risk factors of NEC and predict surgical NEC.

Methods According to the modified Bell's staging criteria, 235 neonates with NEC admitted between Jan 1, 2015 and Sep 30, 2019 were divided into surgical group (n=47) and conservative group (n=188). Data concerning clinical characteristics and therapeutic management were collected. Infection values of WBC, N%, PLT, CRP, PCT were measured before treatment (T0), on the next day after treatment (T1).

Results 1. Surgical group were associated with gestational age, small for gestational age, add milk before onset of NEC, hospitalization expense compared to conservative group ($p=0.040, 0.048, 0.045, 0.011$), in addition to N%0, N%1, PLT1, CRP1 and PCT1 ($p=0.049, 0.003, 0.018, <0.001, 0.003$). There was significant difference in NEC among Bell stage I, II and III between surgical group and conservative group ($\chi^2=49.358, p<0.001$). 2. Regression analysis showed that Bell stage I, II and III NEC (OR 12.331, 6.315, $p=0.038, 0.025$), N%1 (OR 0.959, $p=0.032$), CRP1 (OR 0.978, $p=0.039$) and PCT1 (OR 0.983, $p=0.036$), add milk before onset of NEC (OR 7.117, $p=0.021$) were all independent risk factors to predict surgical NEC. 3. The AUC of combined Bell stage I, II and III NEC, N%1, CRP1, PCT1, add milk the day before onset of NEC (P) in predicting surgical NEC was 0.924 (95%CI 0.865~0.983, $p<0.001$). The ideal cutoff value of P was 0.729, with a sensitivity of 85.7% and specificity of 87.5%.

Conclusion Increased Levels of CRP1, PCT1, N%1, Bell stage I, II and III, and add milk the day before onset of NEC are all independent risk factors of NEC, and combined of them can be used to predict the development of surgical NEC.

Background

Necrotizing enterocolitis (NEC) is a common critical disease of the gastrointestinal system in neonates. Some severe cases can occur respiratory circulation failure, shock, DIC, perforation of the digestive tract, and some important organs involved, and even a threat to life.¹ Niemarkt et al and Houben et al. reported that the mortality rate of NEC can even rise to 15%–30%.¹⁻² Up to 20% to 40% of infants with NEC will need surgical intervention at some point.¹ However, early diagnosis and accurately estimating the day that NEC cases will deteriorate and need surgical intervention are still challenging all over the world.¹ Besides, a study by Ahle et al. in Sweden with 320 NEC and 3567 controls, found independent negative associations with NEC for maternal weight, preeclampsia, maternal urinary infection, premature rupture of the membranes, and birthweight, which suggested that NEC is a multifactorial disease.³ Gordon et al⁴ in American studied a total of 4059 survivors and 1107 infants who died with a diagnosis of medical or surgical NEC, and found that mortality were associated with low gestational age, low platelet count, low hematocrit, high band/segmented neutrophil ratio, earlier day of diagnosis, high birth weight z-score, non-white race, no antenatal steroids in gestations above 24 weeks, absolute lymphocyte count adjusted for gestational age, and absolute monocyte count high and low values. Thus, studying clinical predictive maternal and neonatal factors can contribute to early diagnosis of NEC, and its course and prognosis. The occurrence of NEC is affected by many factors, such as premature intestinal immature, severe bacterial infection, ischemia and hypoxia, intestinal

dysbacteriosis, and even the separation with mother. With continuous improvement of nutrition and feeding strategies for premature infants, severe late onset infection of neonates is significantly more than early infection, and bacterial infection has become the focus of NEC.⁵⁻⁶ Therefore, it is of great clinical significance to discuss C-reactive protein (CRP), procalcitonin (PCT) and other infection indicators in active stage of NEC. Recently, a research by Xia et al on 206 neonates with gastrointestinal perforation in China, found some of them with CRP and PCT rising rapidly in a short time, some with platelet (PLT) decreasing, white blood cell (WBC) rising or dropping, anemia, coagulation dysfunction, suggesting that CRP and PCT and other infection indexes may be associated with gastrointestinal perforation.⁷ Randomized, comparative, multi-center study in Chongqing, China, by Zhang et al reported that CRP-WBC-platelet levels were markedly higher on infants with stricture and elevated levels were maintained until the stricture was healed.⁸ Duci et al in Italy compared 53 patients required surgery with 102 patients who were treated conservatively. Bivariate and multivariate analyses suggested higher CRP levels are prognostic factors associated with the need for surgery.⁹ In our study, 235 neonates with NEC were divided into surgical group and conservative group, and their clinical data and infection indexes were collected and analyzed, respectively. Logistic regression was used to investigate clinical predictors for surgical NEC, and then receiver operating characteristic (ROC) curve was used to further analysis the predictive value for surgery and the best cut-off.

Materials And Methods

Patients population

This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University and, as a retrospective study, data have been managed keeping personal information concealed. All patients with NEC in the neonatal intensive care unit at the First Affiliated Hospital of Anhui Medical University between Jan 1, 2015 and Sep 30, 2019 were reviewed. All patients met criteria of Practice of Neonatology. To preserve diagnostic criteria and reduce missing data, NEC patients submitted from other hospital and other infectious diseases were in excluded. According to modified Bell's criteria¹⁰, 235 neonates with NEC were divided into stage I group (n = 48), stage II group (n = 142), and stage III group (n = 45). According to reference¹⁻³, age of NEC onset was defined as the age of neonates with symptoms of abdominal distension, vomiting, bloody stool, diarrhea. All patients received treatment consisted of intravenous administration of meropenem or cefoperazone sulbactam, metronidazole combined with fluconazole, supplemented by fasting, gastrointestinal decompression, mechanical ventilation, intravenous infusion of gamma globulin or plasma, dopamine and dobutamine to improve circulation, alkaline blood, intravenous nutrition rehydration, and so on.

Definition of Parameters

Peripheral venous blood (2 mL procoagulant tube and 2 mL EDTA anticoagulant) was extracted from each group before treatment (T_0), the next day after treatment (T_1), respectively. XE-2100 fully automatic hematology analyzer and reagent was used to detect blood leukocytes. Vitros-5600 fully automatic biochemical immune analyzer was used to detect CRP, reagent provided by Johnson Company of USA. Mini-

VIDAS-1210 fully automatic immunofluorescence analyzer and reagent from BioMerieux company in America was used to detect PCT.

Statistical Analyses

SPSS 25.0 was used to analyze all data. The measurement data of normal distribution was presented as mean and standard deviation ($\bar{x} \pm s$). Comparison between two groups was done with independent sample t-test. The non-normal distribution data presented as $[P_{50} (P_{25}, P_{75})]$. Mann-Whitney U test was used to compare the two groups. Categorical variable can be described by rate. The χ^2 test was used for comparison between two groups.

Logistic regression was performed to estimate the independent risk factors of surgical NEC. The regression equation was expressed as $\ln(P/(1-P)) = \text{Constant} + B_1\text{CRP} + B_2\text{PCT}$, in which P represented the predicted probability of surgical intervention, B1, B2 represented the logistic regression coefficients of CRP, PCT, respectively. The ROC curve analyses were used to determine the diagnostic utilities of biomarkers. p value ≤ 0.05 was considered statistically significant.

Results

Characteristics of the study group

We identified 235 patients diagnosed with NEC in the study period, 148 males and 87 females. Median gestational age was 32.5 ± 2.6 weeks (range 25–41) and median birth weight was 1703.4 ± 505.3 g (range 690–3700). 81 neonates were very low birth weight infants (VLBW— <1500 g) and 8 neonates were extremely low birth weight (ELBW— <1000 g). Postnatal age at onset NEC was 16.2 ± 11.0 days (range 2–72). 188 patients (80%) were treated conservatively and 47 (20%) required surgical treatment. Among these, 12 underwent surgery due to free intraperitoneal air, 30 due to intestinal stenosis, 5 received only a laparotomy.

Surgical group were associated with gestational age, small for gestational age, add milk before onset of NEC, hospitalization expense compared to conservative group ($p = 0.040, 0.048, 0.045, 0.011$) (Table 1). There was significant difference in NEC among Bell stage I, II and III between surgical group and conservative group ($\chi^2 = 49.358, p < 0.001$) (Table 1).

Table 1
Risk factors of NEC and its course

	Surgical group (n = 47)	Conservative group (n = 188)	$\chi^2/t/z$	P
Gender, male (%)	27(57.4)	121(64.4)	0.771	0.380
BW	1530.0(1317.5, 1822.5)	1400.0(1610.0, 1950.0)	1.625	0.104
Delivery, natural birth (%)	16(22.2)	56(77.8)	0.320	0.571
Gestational age	31.0(30.0, 33.0)	32.0(31.0, 34.0)	2.053	0.040
Small for gestational age (%)	20(42.6)	72(30.6)	3.925	0.048
Premature	43(91.5)	219(93.2)	0.038	0.846
IVF-ET	7(14.9)	41(18.1)	0.266	0.606
GDM n (%)	8(24.2)	25(75.8)	0.432	0.511
Mother age, ($\bar{x} \pm s$)	30.7 $\pm$ 5.7	30.4 $\pm$ 4.9	0.307	0.759
multiply pregnancy	11(23.4)	76(34.6)	2.144	0.143
gestational hypertension	16(34.0)	50(26.6)	1.032	0.310
abnormal gestation and birth	25(53.2)	98(52.1)	0.017	0.896
Bleeding at first PROM	3(6.4)	16(8.5)	0.032	0.858
Bleeding when at birth	48.0(44.0,108.0)	48.0(10.0,96.0)	1.114	0.265
placental abruption	4(8.5)	18(9.6)	1.000	0.541
battledoreplacenta	0(0.0)	1(99.5)	1.000	0.800
velamentous placenta	1(2.1)	0(0.0)	0.200	0.200
placenta previa	0(0.0)	4(2.1)	0.586	0.407
amniotic fluid	2(4.3)	14(7.4)	0.205	0.650
° contamination	3(6.4)	8(4.3)	0.054	0.817
DXM	20(42.6)	77(41.0)	0.040	0.842
feeding intolerance	24(16.9)	118(83.1)	2.153	0.142
add milk before onset of NEC	37(23.7)	119(76.3)	4.009	0.045
use of blood products 1 week before onset of NEC	8(17.0)	19(10.1)	1.768	0.184
Use of PS	8(17.0)	55(29.3)	2.868	0.090
NICU stay $\geq$ 5 day	23(48.9)	103(54.8)	0.518	0.472
Apgar 5 min $\geq$ 7	3(6.4)	8(4.3)	0.050	0.832
Age on NEC (d)	13.0(7.3, 20.0)	14.0(9.0, 20.8)	0.585	0.559
NEC stage, n (%)	0(0.0)	48(100.0)	49.358	$\leq$ 0.001
Bell I	22(15.5)	120(84.5)	2.545	0.011
Bell II	25(55.6)	20(44.4)	0.397	0.691
Bell III	55216.4(31007.6,82279.2)	40634.9(30071.3,57485.1)		
hospital expense	37.0(22.5,56.3)	35.0(27.0,47.3)		
hospital stay				

*Data are presented as mean $\pm$ standard deviation ($\bar{x} \pm s$) or [P₅₀ (P₂₅, P₇₅)].

Laboratory values during the acute phase of NEC were described in the table (Table 2). Before treatment, on the next day after treatment, surgical group had a higher level of N% and N% (p = 0.049, 0.003) than conservative group. On the next day after treatment, surgical group had higher PLT, CRP and PCT than conservative group (p = 0.018, $\leq$ 0.001, 0.003).

Table 2
Infection indexes in surgical group and conservative group at different time points

	Surgical group (n = 47)	Conservative group (n = 188)	t/z	P
WBC0	7.3(4.3, 11.2)	8.2(6.4, 10.5)	1.464	0.143
N%0	61.1 ± 18.5	55.0 ± 18.5	1.982	0.049
PLT0	261.6 ± 108.9	252.9 ± 98.7	0.508	0.612
CRP0	21.9(1.9, 45.9)	8.6(1.8, 34.4)	1.257	0.209
PCT0	2.9(0.3, 9.1)	1.3(0.3, 5.0)	1.208	0.227
WBC1	6.1(3.9, 10.1)	7.5(5.2, 10.3)	1.762	0.078
N%1	52.1 ± 19.6	41.9 ± 15.9	3.063	0.003
PLT1	218.0 ± 110.4	266.4 ± 115.2	2.380	0.018
CRP1	54.1(34.5, 90.8)	15.0(4.1, 35.4)	5.803	0.001
PCT1	9.3(1.6, 56.0)	1.1(0.2, 9.0)	3.845	0.003
*Data are presented as mean ± standard deviation ($\bar{x} \pm s$) or [P ₅₀ (P ₂₅ , P ₇₅)].				

Gestational age, small for gestational age, add milk before onset of NEC, Bell stage I, II and III NEC, N%₀, N%₁, PLT₁, CRP₁ and PCT₁ were enrolled in the logistic regression and the result showed that gestational age, small for gestational age, N%₀, PLT₁ were not statistically significant (p = 0.563, 0.116, 0.054, 0.171). Regression analysis was performed again without gestational age, small for gestational age, N%₀, PLT₁ and showed Bell stage I, II and III NEC (OR 12.331, 6.315, p = 0.038, 0.025), N%₁ (OR 0.959, p = 0.032), CRP₁ (OR 0.978, p = 0.039) and PCT₁ (OR 0.983, p = 0.036), milk before onset of NEC (OR 7.117, p = 0.021) were all independent risk factors to predict surgical NEC (Table 3). The regression equation could be expressed as $\ln(P/(1-P)) = 1.899 + 2.512 \text{ Bell I NEC} + 1.843 \text{ Bell II NEC} - 0.042 \text{ N\%}_1 - 0.022 \text{ CRP}_1 - 0.017 \text{ PCT}_1 + 1.962 \text{ add milk the day before onset of NEC}$.

Table 3
Binary logistic regression for the effects of Bell stage I, II and III NEC, N%₁, CRP₁, PCT₁, add milk the day before onset of NEC in predicting surgical NEC

	OR (95%CI)	P	B	S. E	Wald	df
NEC	1.000	0.038	2.512	1.208	4.325	1
Bell I	12.331(1.156–	0.025	1.843	0.820	5.046	1
Bell II	131.570)	0.032	-0.042	0.020	4.600	1
Bell III	6.315(1.265–31.534)	0.039	-0.022	0.011	4.274	1
N% ₁	0.959(0.923–0.999)	0.036	-0.017	0.008	4.407	1
CRP ₁	0.978(0.958–0.999)	0.021	1.962	0.849	5.344	1
PCT ₁	0.983(0.967–0.999)	0.001	1.899	0.490	15.052	1
Add milk the day before onset of NEC	7.117(1.348–37.574)					
Constant						

The AUC of Combined Bell stage I, II and III NEC, N%₁, CRP₁, PCT₁, add milk the day before onset of NEC (P) in predicting surgical NEC was 0.924 (95%CI 0.865–0.983, p=0.001). The ideal cutoff value of P was 0.729, with a sensitivity of 85.7% and specificity of 87.5%. At a specificity of 100%, the value of P was 0.916.

Discussion

NEC is a severe neonatal intestinal inflammatory disease. Niemarkt et al.¹ and Zhang et al.⁵ reported that 1%~5% of NEC need stay in neonatal intensive care units, and 20%~40% of them need surgical treatment, and the mortality rate of children undergoing surgery is as high as 45%. Yet, clinical and radiologic features are nonspecific and unreliable, and a lack of effective means for early diagnosis of NEC and deciding the optimal timing of operative intervention. According to reference^{1,5,13}, IL-8, IL-33, E-selectin, C5a, and tumor necrosis factor play an important role in pathogenesis of NEC. However, the high-cost and too late result limit their clinical application. Nowadays, more and more research on WBC, PLT, CRP, PCT, and so on had been performed, since blood routine, CRP, PCT can easily be tested and cheap and can quickly get results.^{7-9,13}

In our study, we found that $N\%_1$, CRP_1 , and PCT_1 are independent risk factors of surgical NEC. It means that on the next day after treatment, increasing of $N\%$, CRP, and PCT can predict the need for surgery of NEC. In fact, we have already got some findings on infection index recent years. A research by Yang et al reported that neutrophil/lymphocyte ratio has a good correlation with NEC stage II and stage III, and has higher sensitivity, specificity and Youden index when compared with WBC count, CRP, and pre-albumin, suggested that $N\%$ correlates well with NEC.¹⁴ A study by Zhang et al. reported that first PCT (onset of symptoms) (RR, 1.82; 95% CI, 0.98–3.15; $p = 0.009$) was the independent predictive factors for the post-NEC strictures, showing that PCT involved in the process of the occurrence and development of NEC.⁸ A stepwise forward multivariate logistic regression on 64 SGA infants with Bell's stage II NEC, which showed that elevation of CRP in NEC diagnosis (OR 39.21, 95% CI 6.62–249.2) has an increased risk for deteriorating Bell's stage II NEC, indicated that changes of levels of CRP can identify deterioration of NEC.¹⁵ Thus, $N\%_1$, CRP_1 , and PCT_1 can be used as indicator of occurrence and deterioration of NEC. What's more, in our study we first found add milk the day before onset of NEC associated with surgical NEC, and hospitalization expense was higher in surgical group than conservative group.

Recent years, early diagnosis of NEC and how to predict the deterioration and surgery have become a new research hotspot. A research by Houben et al.² reported that 67 infants in England had an operation for NEC, and their median peak CRP level was 73.1 (range 25.2–232) mg/dl. In our study, we have found levels of CRP and PCT and their changes can help with the early diagnosis of Bell stage II/III NEC, and CRP before treatment and on the next day after treatment had a value in predicting the need for surgery. The AUC were 0.818 and 0.830, and the best cut-off were 14.6 mg/L and 42.9 mg/L, respectively.¹⁶ A study by Yu et al. reported that combination of WBC and PLT at the time when the decision of surgical intervention was made because of clinical deterioration, or 36 h after onset of clinical presentation can predict surgical NEC (AUC = 0.84, 95% CI 0.77–0.92, $p \leq 0.001$).¹³ In our present study, a combination of $N\%_1$, CRP_1 , PCT_1 and adding milk before NEC can predict surgical NEC, the AUC was 0.924, and the best cut-off was 0.729, with a sensitivity of 85.7% and specificity of 87.5%. Sensitivity and specificity are both relatively high level, suggesting that a combination of $N\%_1$, CRP_1 , PCT_1 and adding milk before NEC had high value in the diagnosis of surgical NEC.

In conclusion, $N\%_1$, CRP_1 , PCT_1 , and add milk the day before onset of NEC are all independent risk factors of surgical NEC, and a combination of them can be used for predicting surgery in NEC.

Abbreviations

NEC: Necrotizing enterocolitis; CRP: C-reactive protein; PCT: Procalcitonin; PLT: Platelet; WBC: White blood cell; ROC: Receiver operating characteristic.

Declarations

Ethics approval and consent to participate

The study protocol was approval by the Research Ethics Commission of the First Affiliated Hospital of Anhui Medical University. The permission letter that allowed access to the medical records for this study was obtained from the hospital management office.

Consent for publication

The patients' guardians have consented to submission of their case reports to the journal, and we have obtained written informed consent.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflict of interest.

Funding

No specific funding was provided for this study.

Author's contributions

LW contributed to conception of the manuscript and drafted the manuscript. JWH contributed to the obtaining and interpreting of the clinical information. LLW contributed the conception of the manuscript, and made substantial contribution to manuscript revision. All the authors have read and approved the manuscript for publication and agreed to be accountable for all aspects of the work.

References

- [1] Niemarkt HJ, de Meij TG, van de Velde ME, van der Schee MP, van Goudoever JB, Kramer BW, et al. Necrotizing enterocolitis: a clinical review on diagnostic biomarkers and the role of the intestinal microbiota. *Inflamm Bowel Dis* 2015;21(2): 436-44.
- [2] Houben CH, Chan KW, Mou JW, Tam YH, Lee KH. Management of intestinal strictures post conservative treatment of necrotizing enterocolitis: The long term outcome. *J Neonatal Surg* 2016;5(3):28.
- [3] Ahle M, Drott P, Elfvin A, Andersson RE. Maternal, fetal and perinatal factors associated with necrotizing enterocolitis in Sweden. A national case-control study. *PLoS One* 2018;13(3):e0194352.
- [4] Gordon PV, Swanson JR, Clark R, Spitzer A. The complete blood cell count in a refined cohort of preterm NEC—the importance of gestational age and day of diagnosis when using the CBC to estimate mortality. *J Perinatol* 2016;36(2):121-5.
- [5] Zhang HY, Wang F, Feng JX. Intestinal microcirculatory dysfunction and neonatal necrotizing enterocolitis. *Chin Med J(Engl)* 2013;126(9): 1771-8.
- [6] Lean WL, Kamlin CO, Garland SM, Jacobs SE. Stable rates of neonatal sepsis in a tertiary neonatal unit. *J Paediatr Child Health* 2015;51(3): 294-9.
- [7] Xia RP, Li BX, Zhou CG, Wang HY, Xu G, Zhou CJ, et al. Etiology and prognosis of neonatal gastrointestinal perforation:an analysis of 206 cases. [Chinese Journal of Neonatology](#) 2017;32(1):31-34.
- [8] Zhang H, Chen J, Wang Y, Deng C, Li L, Guo C. Predictive factors and clinical practice profile for strictures post-necrotising enterocolitis. *Medicine (Baltimore)* 2017;96(10):e6273.
- [9] Duci M, Fascetti-Leon F, Erculiani M, Priante E, Cavicchiolo ME, Verlato G, et al. Neonatal independent predictors of severe NEC. *Pediatr Surg Int* 2018;34(6):663-9.
- [10] Walsh MC, Kiegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin N Am* 1986;33:179–201.
- [13] Yu M, Liu G, Feng Z, Huang L. Combination of plasma white blood cell count, platelet count and C-reactive protein level for identifying surgical necrotizing enterocolitis in preterm infants without pneumoperitoneum. *Pediatr Surg Int* 2018;34(9):945-50.
- [14] Yang Y, Cao ZL, Zhou XY, Chen XQ, Pan JJ, Cheng R. Does neutrophil/lymphocyte ratio have good diagnostic value in neonatal necrotizing colitis? *J Matern Fetal Neonatal Med* 2019;32(18):3026-33.
- [15] Luo L, Dong W, Zhang L, Zhai X, Li Q, Lei X. Correlative Factors of the Deterioration of Necrotizing Enterocolitis in Small for Gestational Age Newborns. *Sci Rep* 2018;8(1):13.
- [16] Wang L, Ni SW, Zhu KR, Zhou DY, Wang Y, Wang LL. Changes in C-reactive protein and procalcitonin levels in neonates with necrotizing enterocolitis and their clinical significance. *Zhongguo Dang Dai Er Ke Za*