

# Biomarkers in the Severity of Necrotizing Enterocolitis in Preterm Infants: A Pilot Study

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**Background:** The occurrence of necrotizing enterocolitis (NEC) is a common and severe disease of the digestive system in neonates. This study aims to assess the value of the intestinal tissue oxygen saturation ( $r_{\text{int}}\text{SO}_2$ ) combined with the levels of procalcitonin (PCT) and mean platelet volume (MPV) in predicting the severity of NEC in preterm infants.

**Methods:** This experiment was a retrospective cohort study conducted in the Department of Pediatrics, Hongqi Hospital Affiliated to Mudanjiang Medical University between January 2017 and July 2022. Premature neonates with NEC were enrolled and divided into mild-moderate NEC group and severe NEC group according to Bell's stage. The general information data,  $r_{\text{int}}\text{SO}_2$  and blood parameters such as the white blood cell (WBC) count, platelet count (PLT), PCT, MPV, red blood cell distribution width (RDW), hemoglobin (Hb), C-reactive protein (CRP) were compared between the two groups.

**Results:** A total of 122 patients were enrolled, including 79 mild-moderate NEC and 43 severe NEC. The  $r_{\text{int}}\text{SO}_2$  was lower in severe group than in mild-moderate group ( $P = 0.042$ ), the PCT and MPV were both higher in severe group than in mild-moderate group ( $P = 0.048$ ,  $P = 0.049$ ). The results of logistic regression suggested that the  $r_{\text{int}}\text{SO}_2$  (OR = 1.491,  $P = 0.003$ ), PCT (OR = 3.071,  $P = 0.001$ ) and MPV (OR = 4.027,  $P = 0.015$ ) were independent predictive factors for severity of NEC. The area under the curve (AUC) of the  $r_{\text{int}}\text{SO}_2$  combined with PCT and MPV showed good diagnostic ability in the severity of NEC.

**Conclusion:** The  $r_{\text{int}}\text{SO}_2$  combined with PCT and MPV may be considered as the early biomarkers in the severity of NEC and could help us to diagnose the case early with early treatment with better prognosis.

**Keywords:** premature infants, necrotizing enterocolitis, intestinal tissue oxygen saturation, procalcitonin, mean platelet volume

## Introduction

Necrotizing enterocolitis (NEC) is considered to be a common serious disease of the digestive system and an important cause of serious neonatal complication and death in neonates.<sup>1,2</sup> Therefore, its early diagnosis and appropriate treatment are important. Generally, the severity of NEC is based on clinical and biochemical variables representing the infant's intestinal and systemic condition, whereby the patient characteristics and the comorbidities of interest include post-menstrual age, weight, etc.<sup>3</sup> But there is still a lack of indicators that can aid in the prediction of the severity of NEC.

The intestinal tissue oxygen saturation ( $r_{\text{int}}\text{SO}_2$ ) is documented an important function in gastrointestinal damage and affection of the mucosa of the digestive tract in NEC.<sup>4</sup> The near-infrared spectroscopy (NIRS) is a noninvasive tool used to identify preterm infants at risk of developing NEC or progression to complicated NEC.<sup>5-8</sup> The technique measures  $r_{\text{int}}\text{SO}_2$  to assess end-organ perfusion.<sup>9-11</sup> Serum procalcitonin (PCT), a kind of the calcitonin precursor, is an important mediator produced by monocytes. It starts to increase within 2-3 hours following the affection by the bacterial endotoxin as most of the biomarkers need several hours or even days to rise in response to bacterial toxin, so 3 hours in PCT is a convenient and sensitive index for clinical application.<sup>12-14</sup> The mean platelet volume (MPV) describes the average size of platelets in a blood sample and is a simple, economical and useful diagnostic marker for children.

In light of the previous studies, we aimed to predict rapid suspicion of the severity of NEC through determination of the  $r_{\text{int}}\text{SO}_2$ , serum levels of PCT and MPV.

## Materials and Methods

### Sample Selection

This retrospective study was conducted from January 2017 to July 2022 at the Department of Pediatrics of Hongqi Hospital Affiliated to Mudanjiang Medical University. A request of waiver of written consent was approved by the Ethical Committee of the Hongqi Hospital (2023049) after declaring that the data collection tool is anonymous and does not require collection of any identification data. All procedures were carried out according to the Declaration of Helsinki.

All data were accessed from the patients' electronic medical records. In our study, the mild-moderate NEC was defined as Bell's stage I and II, and the severe NEC was defined as Bell's stage III.<sup>15</sup> None of the included cases used probiotics. The exclusion criteria were as follows: (1) gestational age (GA)  $\geq$  38 weeks; (2) Congenital intestinal tract developmental malformations, inherited metabolic diseases, chromosomal diseases, severe asphyxia, severe congenital heart disease and other diseases; (3) Abdominal skin was damaged.<sup>16</sup>

### Clinical Data and Laboratory Parameters

The patients' records were reviewed to collect the following data on a standardized form: GA, gender, birth weight, mode of delivery, comorbidities during pregnancy, postnatal age, and weight at the time of NEC diagnosis, etc.

The  $r_{\text{int}}\text{SO}_2$  values were obtained within 24 hours after the diagnosis of NEC and continued for 3 hours. For the purpose of routine clinical monitoring of the patients at our department, we used the MNIR-P100 NIRS (Mingxi Medical Equipment, Chongqing, China) in combination with neonatal SomaSensors (Suzhou AIQIN Bio-Medical Electronics, Suzhou, China). The instrument measured  $r\text{SO}_2$  every 5 seconds (0.2 Hz). The intestinal sensor was placed 0.5 to 1 cm below the umbilicus on the central abdomen and sheltered from light. Mepitel film (Mölnlycke, Sweden) was used below each sensor for skin protection and there was no adverse effects on NIRS integrity and validity in the previous research.<sup>11,17</sup> Sensor placement was checked and documented several times a day by the nurse.

Blood samples were collected within the first time after the onset of clinical manifestations of NEC such as bloody stools, bloating. Laboratory parameters such as the white blood cell (WBC), platelet count (PLT), PCT, MPV, red blood cell distribution width (RDW), hemoglobin (Hb), C-reactive protein (CRP) and were measured.

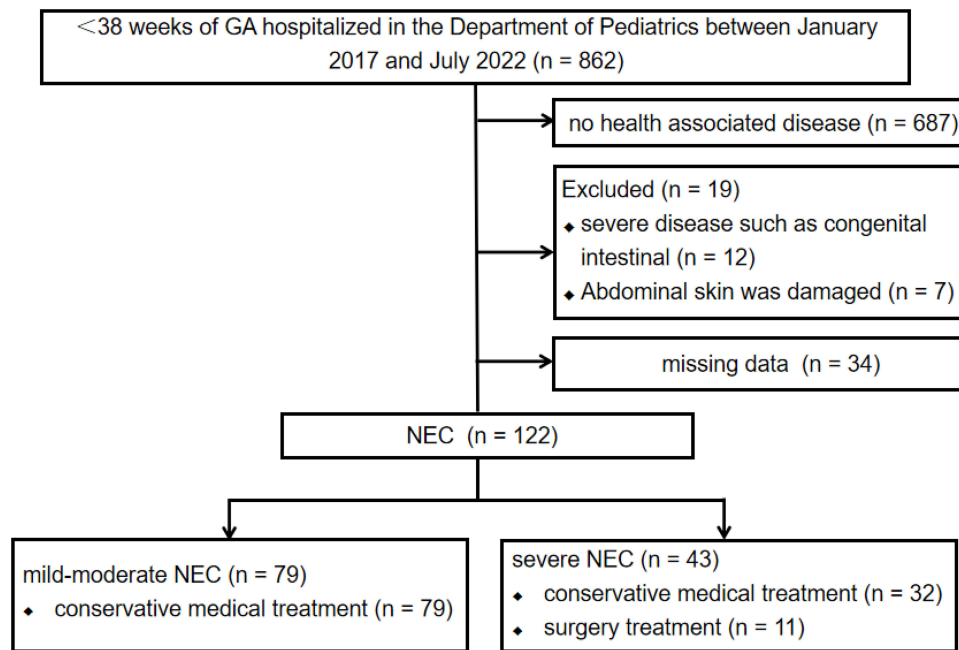
### Statistical Analysis

Statistical analysis was performed using SPSS19.0 software (SPSS, Chicago, USA). Categorical variables are expressed as proportions and were compared using the chi-square ( $\chi^2$ ) test and Fisher's exact test. Continuous variables with a non normal distribution were presented as the median and interquartile range (IQR) percentiles, and variables with a normal distribution were expressed as mean  $\pm$  standard deviation (s.d.). The results of the parameters were compared using *t*-tests or Mann-Whitney *U*-tests. Logistic regression analysis was applied to assess the independent risk factors for the severity of NEC. The receiver operating characteristic (ROC) method was conducted to evaluate the utility of different variables in predicting the severity of NEC. The areas under the ROC curve (AUCs) and optimal cut-off points based on maximizing the sum of the sensitivity and specificity were calculated for each of the variables. *P*-value  $<$  0.05 was considered significant.

## Results

### Patient Characteristics

Out of 862 premature infants hospitalized during the study period, 687 premature infants were identified with no health associated disease. Nineteen patients were excluded because of severe disease and abdominal skin damaged and 34 patients were excluded for missing data on  $r_{\text{int}}\text{SO}_2$  or blood parameters. A total of 122 premature infants were enrolled in the study, including 79 mild-moderate NEC patients and 43 severe NEC patients. Eleven patients were treated with surgery in the severe group, and others and the mild-moderate patients were accepted conservative medical treatment (Figure 1).



**Figure 1** Flow chart of the study.

We analyzed the baseline characteristics of patients with NEC, including their gender, gestational age (GA), < 28 weeks gestation, birth weight, age of onset, breastfeeding, delivery mode, premature rupture of membranes (PROM), maternal hypertension, maternal diabetes, Intra-uterine growth retardation (IUGR). No significant differences were observed regarding these data between the mild-moderate group and the severe group ( $P > 0.05$ , Table 1).

## Clinical Indicator Observations

The  $r_{\text{int}}\text{SO}_2$  were lower in the severe NEC group than the mild-moderate NEC group, but the PCT and MPV were higher in the former ( $P = 0.042$ ,  $P = 0.048$  and  $P = 0.049$ ). No significant differences in WBC, PLT, RDW, Hb and CRP were observed between the two groups ( $P > 0.05$ ) (Table 2).

**Table 1** The Baseline Clinical Characteristics of the Mild-Moderate and Severe NEC Groups

	<b>Mild-Moderate NEC (n = 79)</b>	<b>Severe NEC (n = 43)</b>	<b>P</b>
Gender (male %)	36 (46%)	22 (52%)	0.126
GA (weeks)	33.2 ± 4	31 ± 2	0.087
< 28 weeks gestation	7 (8.9%)	5 (11.6%)	0.383
Birth weight (g)	2165 ± 648	1968 ± 791	0.061
Age of onset (days)	15.26 (4.25–19.75)	14.98 (4–18.5)	0.253
Breastfeeding (%)	41 (52%)	19 (44%)	0.108
Cesarean section (%)	57 (72%)	35 (81%)	0.102
PROM > 18 h (%)	8 (10%)	3 (7%)	0.229
Maternal hypertension (%)	7 (8.9%)	5 (11.6%)	0.362
Maternal diabetes (%)	17 (21.5%)	11 (25.6%)	0.375
IUGR	4 (11.4%)	9 (20.9%)	0.123

**Table 2** Levels of  $r_{\text{int}}\text{SO}_2$ , WBC, PLT, PCT, MPV, RDW, Hb, CRP in Mild-Moderate NEC and Severe NEC Groups (\* $P < 0.05$ )

	Mild-Moderate NEC (n = 79)	Severe NEC (n = 43)	P
$r_{\text{int}}\text{SO}_2$	51.04 ± 4.1	39.56 ± 2.33	0.042*
WBC ( $\times 10^9/\text{L}$ )	10.26 ± 0.97	8.32 ± 1.39	0.069
PLT ( $\times 10^9/\text{L}$ )	297.94 ± 58.93	254.33 ± 73.21	0.172
PCT (ng/mL)	5.75 ± 1.05	14.00 ± 0.84	0.048*
MPV (fL)	9.21 ± 0.60	11.56 ± 0.76	0.049*
RDW (%)	15.37 ± 2.11	14.66 ± 3.53	0.229
Hb (g/L)	127.81 ± 23.44	119.75 ± 22.80	0.924
CRP (mg/L)	21.86 ± 4.88	29.96 ± 5.27	0.326

## Logistic Regression Analysis

The results of logistic regression suggested that the  $r_{\text{int}}\text{SO}_2$  (OR = 1.491,  $P = 0.003$ ), PCT (OR = 3.071,  $P = 0.001$ ) and MPV (OR = 4.027,  $P = 0.015$ ) were independent predictive factors for the severity of NEC (Table 3).

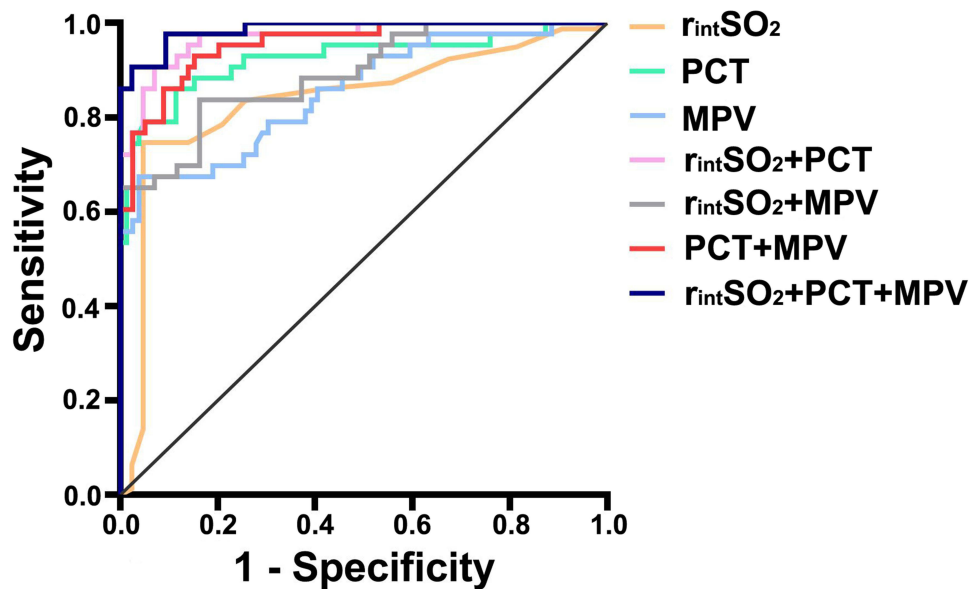
The receiver operating characteristic (ROC) analysis showed that the area under the curve (AUC) was 0.749 for  $r_{\text{int}}\text{SO}_2$  alone, 0.919 for PCT alone, 0.850 for MPV alone, and 0.970 for  $r_{\text{int}}\text{SO}_2$  combined with PCT, 0.892 for  $r_{\text{int}}\text{SO}_2$  combined with MPV, 0.935 for PCT+MPV, and 0.986 for  $r_{\text{int}}\text{SO}_2$ +PCT+MPV, suggesting that  $r_{\text{int}}\text{SO}_2$  combined with PCT and MPV was better for predicting the severity of NEC in preterm infants (Table 4, Figure 2).

**Table 3** Logistic Regression Analysis Results (\* $P < 0.05$ )

	B	S.E	Walds	P	OR	95% CI for OR
$r_{\text{int}}\text{SO}_2$	0.399	0.132	9.074	0.003*	1.491	44.04–46.15
PCT	1.122	0.332	11.415	0.001*	3.071	7.93–9.39
MPV	1.393	0.573	5.904	0.015*	4.027	9.73–10.21

**Table 4** The AUC of  $r_{\text{int}}\text{SO}_2$ , PCT Combined with MPV (\* $P < 0.05$ , \*\* $P < 0.01$ )

	AUC	P	Cut-off Value	Sensitivity	Specificity	Youden Index
$r_{\text{int}}\text{SO}_2$	0.749	0.009*	45.5	0.953	0.747	0.7
PCT	0.919	0.000**	7.635	0.886	0.86	0.746
MPV	0.850	0.000**	9.395	0.962	0.326	0.674
$r_{\text{int}}\text{SO}_2$ +PCT	0.970	0.000**	0.459	0.93	0.907	0.837
$r_{\text{int}}\text{SO}_2$ +MPV	0.892	0.000**	0.424	0.837	0.837	0.674
PCT+MPV	0.935	0.000**	0.221	0.848	0.93	0.778
$r_{\text{int}}\text{SO}_2$ +PCT+MPV	0.986	0.000**	0.731	0.977	0.907	0.884



**Figure 2** The ROC curve of  $r_{\text{intSO}_2}$ , PCT Combined with MPV for predicting the severity of NEC.

## Discussion

NEC is the most common and aggressive gastrointestinal emergency in neonates. Clinical features of NEC are complex and there is a lack of specific biomarkers corresponding to the development of the disease. It means that determining the severity of NEC in time and administering appropriate treatment is difficult and of utmost importance to physicians, parents and infants. Therefore, our research focuses on searching for effective biomarkers that can predict the severity of NEC among commonly used clinical indicators.

NIRS can analyze the ischemic-hypoxic injury which cannot be identified by traditional hemodynamic examination. It reflects the circulatory perfusion of specific organs, and guides the treatment with the goal of oxygen supply and restoration of organ blood flow. In previous studies, most of the current research on NIRS focused on the measurement of cerebral oxygen saturation ( $r_c\text{SO}_2$ ). It dynamic monitored the value every week after birth is basically used to predict the development of late NEC, and the time of each monitoring is short.<sup>5,17–19</sup> There are few studies on dynamic monitoring of  $r_{\text{intSO}_2}$  in preterm infants with definite the severity of NEC. In our study, the  $r_{\text{intSO}_2}$  was lower in the severe NEC group than mild-moderate NEC group at the early stage.

The PCT is a non-active procalcitonin peptide substance, which can predict the status of bacterial infection. The level of PCT is positively correlated with the degree of infection, so it is important for the judgment and prevention of inflammatory diseases.<sup>12</sup> Cai et al<sup>16,20</sup> showed that the serum PCT level of neonates with NEC was significantly higher than that of healthy neonates, and the level of PCT in stage III NEC was significantly higher than stage II NEC, and that in stage II NEC was significantly higher than stage I NEC. Our study found that PCT in the severe group was higher than that in the mild-moderate group. It was agreement with the results in the literature.

The MPV is an easy-to-measure surrogate for platelet activation that is provoked by inflammation.<sup>15</sup> It describes platelet size and platelet production rate in a blood, and increased in the development of platelet-activated diseases and participates in the oxidative process related to inflammation. High MPV and the associated increase in platelet mass has been shown to promote ductus arteriosus thrombosis and anatomic closure in preterm infants.<sup>14</sup> Cekmez et al<sup>21</sup> noted that infants who went on to develop NEC had higher mean platelet volumes, possibly reflecting increased thrombopoiesis. In our study that MPV in the severe group was higher than that in the mild-moderate group. It may be possible that platelet activating factor was enhanced release and lead to platelet aggregation and microthrombus formation, leading to ischemic necrosis of the mesenteric arteries in the case of prematurity, infection, and hypoxic ischemia. At the same time, it promoted the inflammatory reaction.

However, to date, there are but limited data on their relationship with the severity of NEC. We found the result of the ROC curve analysis showed that the AUC was 0.986 for  $r_{\text{int}}\text{SO}_2$ +PCT+MPV, indicating that the biomarkers were very effective in judging the severity of NEC. To further confirm the validity, we calculated the Youden Index. The best interception value, sensitivity and specificity of the groups were obtained through the maximum Youden index. The best cut-off value obtained by the maximum Youden index (0.884) is 0.731 (sensitivity 97.7%; specificity 90.7%). It means  $r_{\text{int}}\text{SO}_2$  combined with PCT and MPV was better at predicting the severity of NEC in preterm infants. They are the simple, economical and useful diagnostic markers for children in the clinical.

## Limitation

Our study suffers from several limitations. Firstly, the study is retrospective in design and limited to the experience of a single center what were obtained by the clinical doctors. The second limitation is that, although our study included more participants than most other studies on this subject, the sample size remains small.

## Conclusion

In summary, our results showed that  $r_{\text{int}}\text{SO}_2$  combined with PCT and MPV could be considered an early biomarkers for diagnosed the severity of NEC in preterm infants. However, the value of  $r_{\text{int}}\text{SO}_2$  may change rapidly in the early stage of NEC, our research was focused on within 24 hours. Follow-up multi-time, multi-factor and prospective research can be carried out to further determine its effectiveness.

## Data Sharing Statement

The data and materials collected in this research are available from corresponding author when requested reasonably.

## Author Contributions

All authors contributed significantly to the work that was published, whether this was in the conceptualization, study design, implementation, data collection, analysis, and interpretation or in all of these areas. They all also participated in writing, revising, or critically evaluating the article, gave their final approval for the version that would be published, agreed on the journal to which the article would be submitted, and agreed to be responsible for all aspects of the work.

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## Disclosure

The authors declare that there are no competing interests in this study.

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