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RESEARCH ARTICLE

Qin et al: Biomarkers in post-NEC intestinal stenosis

Predictive biomarkers for post-neonatal necrotizing enterocolitis intestinal stenosis: Role of JMJD3, CRP, and PCT

Tao Qin[1#], Chenxia Rao[2#], Yunsen Deng[3*]

[1]Pediatrics Center, Wenling First People's Hospital, Wenling, China;

[2]Emergency Center, Zhongnan Hospital of Wuhan University, Wuhan, China;

[3]Department of Neonatology, Shenzhen Bao'an Shiyan People's Hospital, Shenzhen, China.

*Correspondence to Yunsen Deng: dengyssbsph@ph-edu.cn

[#]Equally contributed to this work: Tao Qin and Chenxia Rao.

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ABSTRACT

Necrotizing enterocolitis (NEC) is a severe, often life-threatening gastrointestinal disease in neonates, predominantly affecting preterm infants, and is frequently complicated by intestinal stenosis—a condition whose nonspecific clinical manifestations make early diagnosis and timely intervention particularly challenging. We aimed to investigate the clinical characteristics of NEC intestinal stenosis and its correlation with the histone demethylase Jumonji domain-containing protein 3 (JMJD3). A total of 310 children with NEC treated between February 2021 and June 2024 were retrospectively enrolled, categorizing them into an NEC group (n=265) and a post-NEC intestinal stenosis group (n=45). General data and laboratory indicators were collected, and analyses were performed to identify factors influencing the development of post-NEC intestinal stenosis. Spearman correlation analysis was utilized to assess relationships between JMJD3 and clinical parameters. Results indicated that the post-NEC intestinal stenosis group exhibited significantly lower platelet counts (PLT) and elevated levels of serum C-reactive protein (CRP), procalcitonin (PCT), and JMJD3 in intestinal tissues (p<0.05). JMJD3 was significantly associated with the development of post-NEC intestinal stenosis (p<0.001), presenting a 3.114-fold increased risk. Furthermore, JMJD3 levels were negatively correlated with PLT levels and positively correlated with CRP and PCT levels (p<0.001). Receiver operating characteristic curve analysis demonstrated that the combination of CRP, PCT, and JMJD3 provided the highest predictive efficiency, with an area under the curve of 0.918, sensitivity of 86.67%, specificity of 86.42%, and a Youden index of 0.731 (p<0.05), all surpassing the performance of individual markers. In conclusion, levels of CRP, PCT, and JMJD3 were significantly elevated in NEC children with intestinal stenosis. Their combined assessment presents a highly effective approach for the early diagnosis of post-NEC intestinal stenosis.

Keywords: Intestinal stenosis, necrotizing enterocolitis, CRP, PCT, JMJD3.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a necrotizing intestinal disease in neonates caused by infections from various etiologies and predominantly affects preterm infants. It is not only a common complication in this population but also the most frequent gastrointestinal emergency encountered in the neonatal intensive care unit (NICU) (1, 2). The incidence of NEC ranges from approximately 6% to 15%, with a mortality rate of 30% to 50%, making it a major contributor to neonatal morbidity and mortality (3, 4). NEC can lead to intestinal necrosis or perforation, often resulting in a range of complications, among which intestinal stenosis is one of the most prevalent (5). The pathogenesis of post-NEC intestinal stenosis remains incompletely understood, but it is believed to be associated with ischemia, necrosis, and injury of the intestinal wall secondary to NEC (6). Early diagnosis of intestinal stenosis is challenging, which often leads to clinical misdiagnosis or delayed recognition. Consequently, the growth and development and the quality of life of children are severely affected. Although advancements in early diagnosis and timely surgical interventions (enterectomy and anastomosis or enterostomy) have significantly reduced the incidence of NEC, the occurrence of subsequent intestinal stenosis and obstruction remains relatively high. Intestinal stenosis, the most common complication following NEC, has an incidence rate of 11-35% (7). In NEC patients managed conservatively with medical treatment, intestinal stenosis is mostly found in the transverse colon and descending colon, possibly due to the vulnerability of the terminals branches of the superior and inferior mesenteric arteries to ischemia injury. In contrast, among NEC patients who underwent surgical intervention, intestinal stenosis tends to occur in the ascending colon, possibly due to the unique anatomical and functional characteristics of the ileocecal junction (8). The non-specific clinical presentation of post-NEC intestinal stenosis, often mimicking feeding intolerance or gastroenteritis, makes early diagnosis difficult and increases the risk of missed or delayed treatment, thereby adversely affecting patient outcomes (9). Hence, identifying the risk factors for post-NEC intestinal stenosis and facilitating early diagnosis and intervention are of great significance for improving prognosis in affected children.

Previous study has shown that intestinal inflammatory mechanisms are intricately associated with complex molecular regulatory networks, including transcriptional modulation pathways (10). Noticeably, histone methylation modifications have gained

increasing attention in recent years for their critical roles in the pathogenesis and progression of various diseases, as research in molecular biology continues to advance. Jumonji domain-containing protein 3 (JMJD3), a histone demethylase, has been reported to be significantly upregulated in inflammatory conditions, such as acute pancreatitis and autoimmune thyroiditis. JMJD3 promotes the expression of pro-inflammatory genes, thereby contributing to the development and exacerbation of inflammatory diseases (11, 12). However, whether JMJD3 serves as a contributing factor in the onset and progression of post-NEC intestinal stenosis remains unclear.

Considering the severity of post-NEC intestinal stenosis and the potential role of JMJD3 in its pathophysiology, the present study was conceived to investigate the clinical characteristics of post-NEC intestinal stenosis as well as their association with JMJD3, aiming to provide new insights and strategies for the early diagnosis, targeted intervention, and prevention of post-NEC intestinal stenosis to improve patient prognosis and quality of life.

MATERIALS AND METHODS

Subjects

A total of 310 NEC children diagnosed with NEC and treated at our hospital between February 2021 and June 2024 were retrospectively enrolled and assigned into either the NEC group or a post-NEC intestinal stenosis group according to the absence or presence of intestinal stenosis.

The inclusion criteria were as follows: 1) children who met the diagnostic and treatment criteria for NEC and were diagnosed with NEC based on clinical symptoms (13), imaging and surgical pathology, 2) those in Bell stage I-III, 3) those completing the diagnosis and treatment of NEC and post-NEC intestinal stenosis at our hospital, and 4) those offering complete clinical case data. Exclusion criteria included: 1) children with congenital dysplasia of the heart, liver, kidneys, or intestinal system, or 2) those with intestinal atresia, omphalocele, megacolon or other gastrointestinal diseases. The study was approved by the Institutional Review Board of Wenling First People's Hospital (NO. ZJWL-F202112).

Acquisition of general data

Data were collected on maternal factors, including gestational hypertension, gestational diabetes, mode of conception (natural conception or *in vitro* fertilization), and premature rupture of membranes and neonatal factors, including mode of delivery (natural delivery or cesarean delivery), gender, gestational age, premature birth, birth weight, antibiotic use, hypoproteinemia, and history of blood transfusion.

Detection of laboratory indicators

A total of venous blood (2 mL) was collected from each child, and centrifuged using a high-speed centrifuge [Optima XPN, Beckman Coulter International Trading (Shanghai) Co., Ltd.]. The supernatant was then harvested for subsequent analyses. Serum white blood cell count (WBC), platelet count (PLT) and neutrophil ratio (NE) were measured using an automatic hematology analyzer (MEK-7222K, Shanghai Jumu Medical Instrument Co., Ltd., China). Serum albumin (ALB) level was detected with an automatic biochemical analyzer (PUZS-300, Tips Biological, Shanghai, China) using a commercial kit [bought from Shanghai Ze Ye Biological Technology Co., Ltd., China (catalogue number: ZY-ALB-Hu)].

Serum levels of C-reactive protein (CRP) and procalcitonin (PCT) were detected through enzyme-linked immunosorbent assay (ELISA) using kits from Bioleaper, Shanghai, China (catalogue number: BR6000016) and Bio-Lab, Beijing, China (catalogue number: ARB13082), respectively. For CRP level detection, an appropriate volume of serum was added to each well of a microplate, followed by the addition of the primary antibody (anti-CRP antibody). The plate was incubated for 2 h at room temperature or overnight at 4°C. After washing to remove unbound antibodies, horseradish peroxidase (HRP)-labeled secondary antibody was added and incubated for 1 h at room temperature. Subsequently, 3,3',5,5'-tetramethylbenzidine (TMB) substrate reagent was added and incubated for 30 min at room temperature in the dark. The reaction was terminated by adding stop solution, and the optical density (OD) at 450 nm was measured. For PCT detection, the serum sample was added to a separate microplate well, followed by the incubation with the capture antibody (anti-PCT antibody) overnight at 4°C. After washing, HRP-labeled detection antibody was added and incubated for 1 h at room temperature. TMB substrate reagent was then added and incubated for 30 min at room temperature in the dark, followed by the addition of stop solution. The OD value at 450 nm was measured with a fully

automated biochemical analyzer or a microplate reader [purchased from Molecular Devices (Shanghai), model: SpectraMax i3x], and the concentrations of CRP and PCT were calculated according to the standard curves.

Western blotting was performed to assess the expression of JMJD3 in intestinal tissues. For children in the NEC group, intestinal tissues were obtained either: 1) from surgically resected inflammatory necrotic bowel segments during laparotomy in severe NEC cases, or 2) via biopsy of affected intestinal segments in cases requiring endoscopic or operative intervention for diagnosis or management. For NEC patients managed non-surgically (i.e., conservatively), no intestinal samples were available for Western blotting analysis, and these patients were not included in this molecular detection subanalysis to avoid introducing tissue sampling bias. In the post-NEC intestinal stenosis group, intestinal tissues were collected from the surgical resection margin during stenosis corrective surgery. To minimize sampling bias, all tissues were collected intraoperatively under standard procedures and by trained pediatric surgeons. The median time from resection to freezing was consistently maintained within 30 minutes to ensure sample integrity. Furthermore, identical anatomical segments were always sampled to provide consistent data. All tissue samples were immediately snap-frozen in liquid nitrogen after surgical excision and stored at −80°C until protein extraction. Before analysis, the tissues were randomly selected from available samples ensuring representative anatomical locations, and handled by blinded personnel to reduce operator bias.

The total proteins were extracted from intestinal tissues using lysis buffer (RIPA buffer, Shanghai Aladdin Biochemical Technology Co., Ltd., China, catalogue number: R493085), followed by high-speed centrifugation to collect the supernatant. The extracted proteins were then separated by SDS-PAGE (Wanleibio, China, catalogue number: WLA005a)and then transferred to a PVDF membrane. The membrane was then blocked with non-specific antibodies (BSA or skim milk) for 1 h at room temperature to reduce non-specific binding in general. Thereafter, the blocked membrane was incubated with a primary antibody against JMJD3 (Wuhan Booute Biotechnology Co., Ltd., China, catalogue number: orb1460584) overnight at 4°C. On the next day, the membrane was incubated with HRP-labeled secondary antibodies. The protein signals were visualized using an ECL substrate (Bio-Rad Laboratories, catalogue numbers: 1705060, 1705061, and 1705062), and an X-ray film was used to

record the signal intensity of bands. The signal intensity is directly proportional to the expression level of target proteins. Results were often normalized to GAPDH.

Statistical analysis

Statistical analysis was performed with SPSS 24.0 software. The normality of continuous variables was tested using the Shapiro-Wilk test. Normally distributed measurement data were presented as mean ± standard deviation ($\bar{x} \pm s$) and compared using the independent-samples *t*-test between groups. Non-normally distributed data were expressed as median (interquartile range) and analyzed using the Mann-Whitney U test. Count data were presented as [*n* (%)] and analyzed using χ^2 test.

To evaluate the correlation between JMJD3 expression and laboratory indicators, Spearman's rank correlation coefficient (ρ) was used for all variables to ensure robust inference regardless of distribution type.

To identify independent risk factors for post-NEC intestinal stenosis, multivariate logistic regression analysis was conducted. Candidate variables were selected based on clinical relevance and prior literature, including CRP, PCT, JMJD3, and other general or laboratory indicators with $p<0.10$ in univariate analyses. Before inclusion, multicollinearity among predictors was assessed using variance inflation factors (VIF), and variables with VIF>5 were excluded. A backward stepwise logistic regression approach was used (inclusion criterion $p<0.05$, exclusion criterion $p>0.10$). The model was built as Logit(*P*)=constant term + X1 × B1 + X2 × B2 + X3 × B3 + ..., and the odds ratio (OR) and 95% confidence interval (*CI*) were calculated.

Receiver operating characteristic (ROC) curves were plotted to assess the predictive performance of CRP, PCT, JMJD3. For the combined prediction model of CRP, PCT, and JMJD3, a multivariate logistic regression model was constructed to derive a logistic score, which was subsequently used for ROC curve analysis. To evaluate the robustness and potential overfitting of the logistic regression model combining JMJD3, CRP, and PCT, internal validation was performed using bootstrap resampling (1,000 iterations). In each iteration, a bootstrap sample was drawn with replacement, maintaining the original class proportions. The corresponding optimism-corrected AUC, sensitivity, and specificity were computed across all bootstrap iterations and reported as mean values with 95% CIs. $p<0.05$ indicated statistical significance.

RESULTS

General data of NEC children with intestinal stenosis

All indicators of maternal and neonatal conditions showed no statistically significant differences between NEC and post-NEC intestinal stenosis groups (p>0.05) (Table 1).

Laboratory indicators in NEC children with intestinal stenosis

The serum levels of WBC, ALB and NE were comparable between the NEC group and the post-NEC intestinal stenosis group (p>0.05). However, the post-NEC intestinal stenosis group exhibited a significant decrease in the serum PLT and significant elevation in serum levels of CRP, PCT and JMJD3 expression in intestinal tissues compared with the NEC group (p<0.05) (Table 2). As shown in Figure 1, the protein expression of JMJD3 was significantly upregulated in intestinal tissues in post-NEC intestinal stenosis group compared with that in NEC group.

Multivariate analysis of factors associated with post-NEC intestinal stenosis

Multivariate Logistic regression analysis was conducted to identify factors associated with the development of post-NEC intestinal stenosis. A backward stepwise logistic regression approach was applied, and the full list of candidate variables along with their elimination order is exhibited in Table S1 (supplementary material). The results revealed that CRP, PCT and JMJD3 were independent significant indicators (p<0.05). Although PLT showed a trend toward significance (p=0.101), it did not meet the threshold for statistical significance. The elevated levels of CRP (p=0.005) and PCT (P=0.005) were associated with the increased risks of post-NEC intestinal stenosis, corresponding to 2.239-fold and 2.593-fold increases in risk, respectively. JMJD3 demonstrated the strongest association (p<0.001), with its upregulation linked to a 3.114-fold increase in the risk of developing intestinal stenosis (Table 3 and Figure 2). CRP, PCT, and JMJD3 were retained as the independent predictors of post-NEC intestinal stenosis, and the final logistic regression equation was: Logit(P) = -5.321 + 0.806 × CRP + 0.953 × PCT + 1.136 × JMJD3, where P is the predicted probability. Of note, no significant multicollinearity was detected among included variables (all VIF<2.0), supporting the reliability of the logistic regression model.

Results of correlation analysis

According to Spearman's rank correlation analysis, JMJD3 level had a negative correlation with PLT level, as well as positive correlations with CRP and PCT levels (P<0.001) (Table 4).

Efficiency of CRP, PCT, JMJD3 and their combination for predicting the development of post-NEC intestinal stenosis assessed by ROC curve analysis

ROC curve analysis demonstrated that CRP had a relatively strong predictive value for post-NEC intestinal stenosis with an area under the curve (AUC) of 0.789, (95% *CI*: 0.707-0.871), sensitivity of 77.78% (95% *CI*: 63.9–87.7%), specificity of 69.81% (95% *CI*: 59.1–78.7%), Youden index of 0.476, and a cut-off value of 56.28 mg/L (p<0.05). PCT also exhibited good predictive performance, with an AUC of 0.812, (95% *CI*: 0.739-0.884), sensitivity of 77.78% (95% *CI*: 63.9–87.7%), specificity of 71.70% (95% *CI*: 61.1–80.5%), Youden index of 0.495, and a cut-off value of 9.35 μg/L (p<0.05). JMJD3 showed the highest predictive accuracy among the individual biomarkers, with an AUC of 0.878, (95% *CI*: 0.827-0.929), sensitivity of 71.11% (95% *CI*: 56.3–82.6%), specificity of 88.30% (95% *CI*: 79.4–94.1%), Youden index of 0.594, and a cut-off value of 637.14 pg/mL (P<0.05). Notably, the combination of CRP, PCT, and JMJD3 based on the logistic regression model score showed the highest predictive performance (AUC=0.918, 95% *CI*: 0.884-0.952), with a sensitivity of 86.67% (95% *CI*: 73.8–94.2%), specificity of 86.42% (95% *CI*: 77.3–92.7%) and Youden index of 0.731 (p<0.05), outperforming each single biomarker (Table 5 and Figure 3). The logistic regression model combining JMJD3, CRP, and PCT achieved a strong discriminative performance after internal validation. The optimism-corrected AUC based on 1,000 bootstrap iterations was 0.892 (95% CI: 0.845–0.935). When applying the optimal cutoff derived from the Youden index in each bootstrap sample, the corrected sensitivity was 86.8% (95% CI: 68.9–97.8%) and specificity was 81.1% (95% CI: 69.1–93.2%). These findings support the high diagnostic utility of the model for predicting post-NEC intestinal stenosis.

DISCUSSION

NEC is a common acute abdominal disease in newborns and most newborns gradually stabilize after active treatment in the acute phase. However, the incidence rate of intestinal stenosis in NEC patients is increasing every year (7). Identifying the factors

influencing the development of post-NEC intestinal stenosis and performing early prediction and diagnosis as well as timely intervention is crucial to reducing the need for reoperation and improving the long-term prognosis in affected children. In this study, we investigated the clinical and molecular characteristics of post-NEC intestinal stenosis and identified potential predictive biomarkers. Our findings revealed that while general perinatal parameters were comparable between NEC children with and without subsequent intestinal stenosis, notable differences emerged in specific inflammatory and molecular indicators. In particular, the levels of CRP, PCT, and intestinal JMJD3 expression were significantly elevated in the stenosis group, whereas platelet count was reduced, suggesting the involvement of a systemic inflammatory response and epigenetic regulation in the development of post-NEC complications.

It is well-known that low birth weight and premature birth function as major risk factors for the development of post-NEC intestinal stenosis. In the present study, these confounding variables were excluded from the analysis to isolate other potential influencing factors. Through analysis, CRP, PCT, and JMJD3 were identified as independent factors associated with the development of post-NEC intestinal stenosis. Previous research has demonstrated elevated inflammation-related factors in acute NEC, along with persistent intestinal abnormalities, including stenosis, following disease resolution (14). Intestinal stenosis, a hallmark complication of inflammatory intestinal diseases, is thought to result from sustained inflammatory responses and repeated fibrotic remodeling of the intestinal wall (15). CRP, an acute phase protein, is significantly upregulated in response to inflammation, tissue injury, or infections via cytokine-mediated pathways. Higher CRP levels have been associated with more severe inflammatory responses (16). Persistent elevation of CRP and abnormal lactate levels have been reported to be predictors for post-NEC intestinal stenosis, which aligns with our findings (1). Wang *et al*. (17) proposed that excessive inflammation mediated by the release of cytokines and chemokines disrupts intestinal mucosal integrity, impairs mucosal repair, and promotes the progression of NEC, ultimately leading to intestinal necrosis and perforation. PCT, a precursor of calcitonin and a key marker of monocyte activation, rises early during inflammation and serves as a sensitive and practical clinical biomarker (18). In combination with intestinal oxygen saturation and mean platelet volume, serum PCT has demonstrated strong predictive

value for assessing NEC severity (19). Consistent with these observations, our study revealed that CRP and PCT expressions enhanced in post-NEC intestinal stenosis patients, with AUCs of 0.789 for CRP and 0.812 for PCT, indicating moderate to good predictive performance.

Previous study has shown that JMJD3 plays a key role in inflammatory diseases. For instance, JMJD3 expression is increased in LPS-induced RAW264.7 and JMJD3 elevation greatly facilitates the production of pro-inflammatory factors TNF-α, IL-1β and IL-6 (20). In osteoarthritis, JMJD3 expression is enhanced, which can activate NF-κB signaling pathway and thus promote inflammatory response (21). Intriguingly, JMJD3 has been found to regulate intestinal inflammation, particularly during the progression of colitis. It is found that JMJD3 can target Nrf2 to regulate NLRP3 inflammasome activation and aggravate colitis progression in mice induced by dextran sodium sulfate (22). In addition, JMJD3 levels have been found to be raised in NEC patients and neonatal mice subjected to experimental NEC it can promote pro-inflammatory response with increased IL-6 and TNF-α release to drive NEC progression (23). Combined with above findings, it can be easily found that JMJD3 has close involvement in inflammatory intestinal diseases, including NEC. Our data showed that JMJD3 expression was distinctly increased in the intestinal tissues of patients with post-NEC intestinal stenosis, with ROC analysis yielding an AUC of 0.878.

Importantly, combining CRP, PCT, and JMJD3 into a diagnostic panel resulted in an improved AUC of 0.918. While this combination outperformed any individual marker, it is worth noting that the confidence intervals for JMJD3 alone (AUC = 0.878; 95% *CI*: 0.827-0.929) and the combination model (AUC = 0.918; 95% *CI*: 0.884–0.952) overlapped substantially, suggesting only an incremental rather than statistically dramatic gain. Nevertheless, the combination markedly enhanced the Youden index (from 0.594 to 0.731) and improved sensitivity (from 71.11% to 86.67%), which may provide meaningful clinical benefit in real-world settings where higher diagnostic accuracy is critical.

Given its central involvement in inflammatory processes, JMJD3 has attacted attention as a potential therapeutic target (24). Mechanistically, JMJD3 functions as a histone H3K27 demethylase that removes repressive epigenetic marks and promotes

transcription of pro-inflammatory genes (20). Among the most studied pharmacologic inhibitors is GSK-J4, a cell-permeable prodrug that is intracellularly converted to the active JMJD3 inhibitor GSK-J1 (25). GSK-J4 has shown anti-inflammatory efficacy in various preclinical models, including colitis, arthritis, and sepsis, by downregulating IL-6, IL-17 and TNF-α and attenuating inflammasome activation (26, 27). These findings suggested that JMJD3 inhibition may mitigate intestinal inflammation. However, several challenges remain before clinical translation, particularly in neonates (28-30).

This study has limitations. First, although NEC patients were classified according to Bell stage I–III, no stratified analysis was performed to assess JMJD3 expression or other relevant clinical indicators across different disease stages. Secondly, JMJD3 expression was assessed at a single time point, and the lack of a longitudinal evaluation precludes insight into its temporal dynamics during disease progression and resolution. Future studies incorporating serial sampling at multiple stages of NEC and recovery are warranted to clarify the prognostic potential of JMJD3 in stricture development. Besides, potential differences in the role of JMJD3 across diverse ethnic groups and clinical settings warrant further investigation to ensure broader clinical applicability. It is believed that the continuous deepening of research will bring new breakthroughs and hopes for the prevention and treatment of post-NEC intestinal stenosis.

CONCLUSION

In conclusion, CRP, PCT and JMJD3 are significantly upregulated in NEC children with intestinal stenosis, and serve as key risk factors associated with the development of post-NEC intestinal stenosis. Moreover, the combined detection of these three biomarkers demonstrates high diagnostic efficiency for identifying affected individuals. Given the pivotal role of JMJD3 in the pathogenesis of post-NEC intestinal stenosis, it may work as a promising therapeutic target, thereby offering novel insights for future intervention strategies.

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TABLES AND FIGURES WITH LEGENDS

Table 1. General data of NEC children with intestinal stenosis [*n* (%), ($x \pm s$)]

Group	NEC group (*n*=265)	Post-NEC intestinal stenosis group (*n*=45)	t/χ^2	*p*
Maternal condition				
Gestational hypertension	44 (16.60)	8 (17.78)	0.038	0.845
Gestational diabetes	20 (7.55)	4 (8.89)	0.097	0.756
Mode of conception			0.034	0.854
Natural conception	233 (87.92)	40 (88.89)		
In vitro fertilization	32 (12.08)	5 (11.11)		
Premature rupture of membranes	24 (9.06)	3 (6.67)	0.276	0.599
Neonatal condition				
Mode of delivery			1.600	0.206
Natural delivery	115 (43.40)	15 (33.33)		
Cesarean delivery	150 (56.60)	30 (66.67)		
Gender			0.103	0.748
Male	154 (58.11)	25 (55.56)		
Female	111 (41.89)	20 (44.44)		
Gestational age (week)	36.74±3.79	37.01±4.49	0.430	0.668
Premature birth	142 (53.58)	27 (60.00)	0.638	0.424
Birth weight (g)	2714.36±610.58	2728.33±716.03	0.138	0.890
Antibiotic use	164 (61.89)	28 (62.22)	0.002	0.966
Hypoproteinemia	51 (19.25)	7 (15.56)	0.344	0.557
Blood transfusion	194 (73.21)	39 (86.67)	3.733	0.053

Table 2. Laboratory indicators in NEC children with intestinal stenosis ($x \pm s$)

Group	NEC group (n=265)	Post-NEC intestinal stenosis group (n=45)	Z/t	p
WBC ($\times 10^9$/L)	12.10±3.20	12.84±3.74	1.398	0.163
PLT ($\times 10^9$/L)	120.80±39.17	108.23±35.31	2.018	0.045
ALB (g/L)	31.74±2.45	32.13±3.71	0.907	0.365
NE (%)	0.67±0.17	0.65±0.15	0.742	0.459
CRP (mg/L)	48.5 (43.2, 54.8)	73.6 (61.7, 85.9)	6.124	<0.001
PCT (μg/L)	6.1 (5.2, 7.3)	13.2 (10.6, 15.9)	7.052	<0.001
JMJD3 expression	4.47±1.74	8.85±2.36	14.753	<0.001

Table 3. Results of multivariate analysis on the occurrence of post-NEC intestinal stenosis

Indicator	β	Standard error	Wald	p	Odds ratio	95% CI
PLT	-1.041	0.635	2.690	0.101	0.353	0.098-1.272
CRP	0.806	0.273	8.717	0.005	2.239	1.311-3.823
PCT	0.953	0.320	8.869	0.005	2.593	1.385-4.855

JMJD3	1.136	0.341	11.098	<0.001	3.114	1.597-6.074

Table 4. Results of Spearman's rank correlation analysis between laboratory indicators and JMJD3 expression

Indicator	JMJD3	
	ρ	*p*
PLT	-0.583	<0.001
CRP	0.476	<0.001
PCT	0.349	<0.001

Table 5. Efficiency of CRP, PCT, JMJD3 and their combination for predicting the occurrence of post-NEC intestinal stenosis assessed by ROC curve analysis

Indicator	AUC (95% *CI*)	Sensitivity (95% *CI*)	Specificity (95% *CI*)	Youden index	Cut-off value	*p*
CRP	0.789 (0.707-0.871)	77.78% (63.9–87.7%)	69.81% (59.1–78.7%)	0.476	56.28 mg/L	<0.05
PCT	0.812 (0.739-0.884)	77.78% (63.9–87.7%)	71.70% (61.1–80.5%)	0.495	9.35 μg/L	<0.05
JMJD3	0.878 (0.827-0.929)	71.11% (56.3–82.6%)	88.30% (79.4–94.1%)	0.594	637.14 pg/mL	<0.05
Combination	0.918 (0.884-0.952)	86.67% (73.8–94.2%)	86.42% (77.3–92.7%)	0.731	Predicted probability ≥0.63	<0.05

Note: Although the combination model achieved the highest AUC, the 95% confidence intervals of the individual markers, particularly JMJD3, overlap, indicating that the performance gain may be incremental rather than statistically significant.

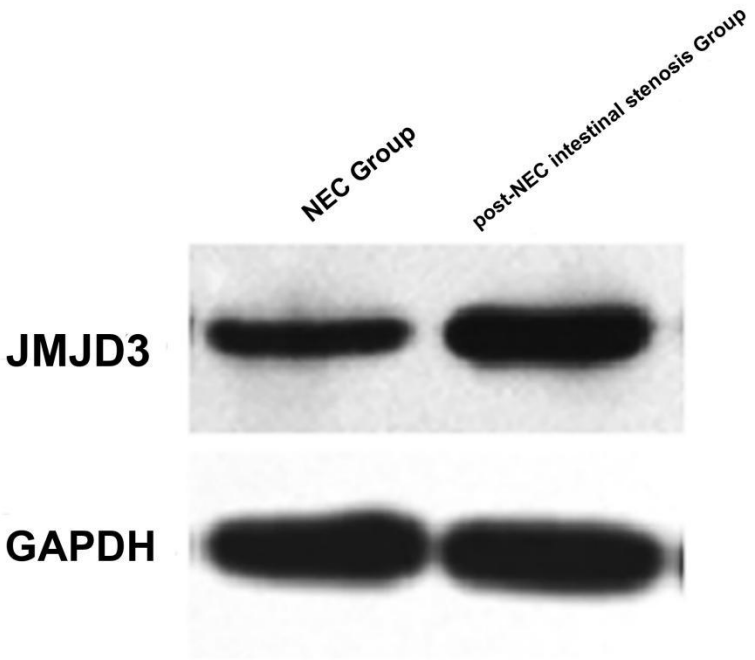

Figure 1. **Upregulation of JMJD3 protein expression in intestinal tissues of post-NEC intestinal stenosis patients compared with NEC group.** GAPDH was used as a loading control. JMJD3 expression was markedly elevated in the post-NEC intestinal stenosis group compared with the NEC group ($p < 0.05$).

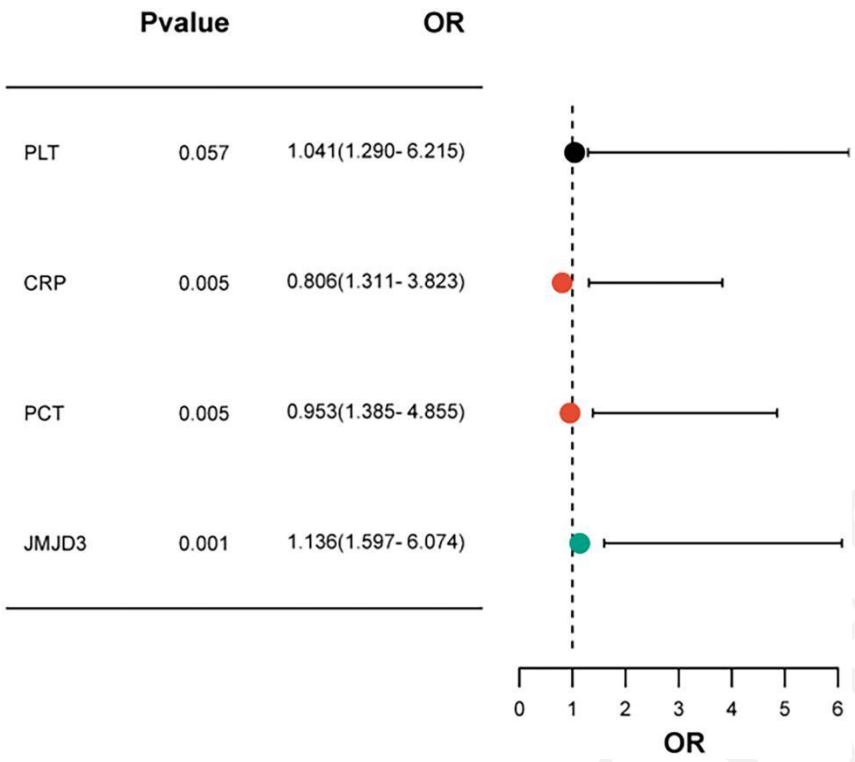

Figure 2. **Forest plot of multivariate analysis on the occurrence of post-NEC intestinal stenosis.** Red dots represent statistically significant predictors (p<0.05), while grey dots denote non-significant predictors (p≥0.05).

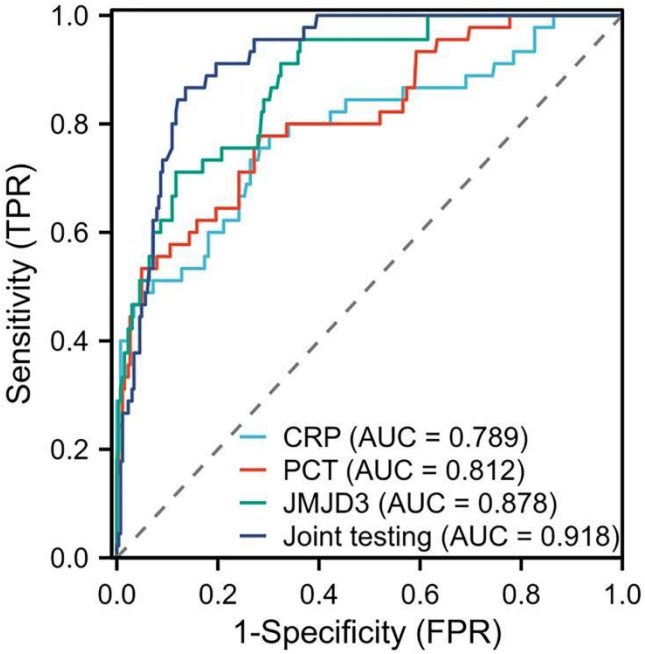

Figure 3. **ROC curve analysis on CRP, PCT, JMJD3 and their combination for predicting the occurrence of post-NEC intestinal stenosis.** ROC curves showing the predictive performance of CRP, PCT, JMJD3, and their combination for the diagnosis of post-neonatal NEC intestinal stenosis. Among individual biomarkers, JMJD3 achieved the highest AUC = 0.878, followed by PCT (AUC = 0.812) and CRP (AUC = 0.789). The combined logistic regression model incorporating CRP, PCT, and JMJD3 demonstrated the greatest predictive accuracy (AUC = 0.918), outperforming each single biomarker. Abbreviations: ROC, receiver operating characteristic; CRP, C-reactive protein; PCT, procalcitonin; NEC, necrotizing enterocolitis; AUC, area under the curve.

SUPPLEMENTAL DATA

Table S1. Stepwise logistic regression process for predicting post-NEC intestinal stenosis

Variable	Univariate *p* value	Stepwise status	β coefficient	Standard error	Wald	*p*	Odds ratio (95% CI)	Retained in final model
Gestational hypertension	0.845	Not entered	-	-	-	-	-	-
Gestational diabetes	0.756	Not entered	-	-	-	-	-	-
Mode of conception	0.854	Not entered	-	-	-	-	-	-
Premature rupture of membranes	0.599	Not entered	-	-	-	-	-	-
Mode of delivery	0.206	Not entered	-	-	-	-	-	-
Gender	0.748	Not entered	-	-	-	-	-	-
Gestational age	0.668	Not entered	-	-	-	-	-	-
Premature birth	0.424	Not entered	-	-	-	-	-	-
Birth weight	0.890	Not entered	-	-	-	-	-	-

Antibiotic use	0.966	Not entered	-	-	-	-	-	-
Hypoproteinemia	0.557	Not entered	-	-	-	-	-	-
Blood transfusion	0.053	Eliminated in stepwise	-	-	-	-	-	No
WBC	0.163	Not entered	-	-	-	-	-	-
PLT	0.045	Trend retained	-1.041	0.635	2.690	0.101	0.353 (0.098-1.272)	No
ALB	0.365	Not entered	-	-	-	-	-	-
NE (%)	0.459	Not entered	-	-	-	-	-	-
CRP	<0.001	Retained	0.806	0.273	8.717	0.005	2.239 (1.311-3.823)	Yes
PCT	<0.001	Retained	0.953	0.320	8.869	0.005	2.593 (1.385-4.855)	Yes
JMJD3	<0.001	Retained	1.136	0.341	11.098	<0.001	3.114 (1.597-6.074)	Yes