Clinical research protocol

Project name: Risk factors for poor prognosis in neonatal necrotizing enterocolitis

Leading unit: Guangzhou Women and Children's Medical Center

Project leader: Wei Zhou

Department: Pediatrics

Research period: July 1, 2024 to June 30, 2025

Project number: EK-NEC20240705

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Catalogue

Abstract

Object name	Analysis of integrated clinical, laboratory and prognostic data of neonates with necrotizing enterocolitis
Goal of study	This retrospective cohort study aims to enroll patients with necrotizing enterocolitis (NEC) from July 1, 2017 to December 31, 2022, conduct a comprehensive analysis of their clinical characteristics, metabolic indicators, laboratory parameters, prognosis, and outcomes, and identify indicators that may predict the progression of neonatal NEC and its associated mortality.
Research design	Retrospective cohort study
Research period	July 1, 2024 to June 30, 2025
Leading unit	Guangzhou Women and Children's Medical Center
Research Consultant	Wei Zhou; Yueju Cai
Research object	Neonates with necrotizing enterocolitis (NEC) from July 1, 2017 to December 31, 2022, conduct a comprehensive analysis of their clinical characteristics, metabolic indicators, laboratory parameters, prognosis, and outcomes.
Inclusion criteria	(1) Diagnosis of NEC
Excluded criteria	Data that meets any of the following conditions need to be eliminated: (1) Congenital gastrointestinal malformations (e.g., intestinal atresia, Hirschsprung's disease) or spontaneous intestinal perforation; (2) Hereditary metabolic disorders; (3) refusal of participation.
Sample size	220 newborns who suffered from necrotizing enterocolitis between

	July 1, 2017 to December 31, 2022
Data collection	Data collection was performed via structured extraction from electronic medical records. The following variables were systematically retrieved: (1) Neonatal medical history, including sex, gestational age, birth weight, and history of birth asphyxia (e.g., Apgar scores); (2) Maternal medical history, particularly mode of delivery, chorioamnionitis, and premature rupture of membranes; (3) NEC-related clinical parameters: Bell's staging, clinical manifestations (abdominal distension, bloody stools, pneumoperitoneum), complications (patent ductus arteriosus [PDA], sepsis, shock), therapeutic interventions (mechanical ventilation, surgical treatment), and mortality; (4) CBC parameters, lactate levels, and parameters required for calculating the nSOFA score at the onset of NEC.
Statistical processing	Statistical analyses and graphic visualization were performed using SPSS 26.0 (IBM Corp.) and GraphPad Prism 9.0. Data for normally distributed, skewed distributed, and categorical variables were expressed as mean ± standard deviation, median (interquartile range), and frequency (%), respectively. Intergroup comparisons of categorical variables were conducted using the Chi-square (χ^2) test or Fisher's exact test, while continuous variables were analyzed using the Student's t-test or Mann-Whitney U test. Variables with $p < 0.05$ in univariate analysis were subsequently included in multivariate logistic regression analysis. Mediation analysis was performed using the PROCESS macro 4.1, with 1000 bootstrap iterations to decompose the total effect into direct and indirect paths. Receiver operating characteristic (ROC) curve analysis was conducted using SPSS 26.0 to evaluate the predictive performance of individual and combined biomarkers. All analyses adopted two-tailed tests, and $p < 0.05$ was considered statistically significant.
Abbreviation	NEC, necrotizing enterocolitis; PNEC, perforated necrotizing

	enterocolitis; VLBW, very low birth weight; SGA, small for gestational age; NICUs, neonatal intensive care units; PDA, patent ductus arteriosus; RDS, Respiratory distress syndrome; CRP, C-reactive protein; WBC, white blood cell; CBC, complete blood count; NEU, absolute neutrophil count; LYM, absolute lymphocyte count; MON, absolute monocyte count; EOS, eosinophilic granulocyte; RBC: red blood cell; PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; NLR, neutrophil-to-lymphocyte ratio; NMR, neutrophil-to-monocyte ratio; MLR, monocyte-to-lymphocyte ratio; SII, systemic inflammatory index = (NEU*PLT)/LYM; SIRI, systemic inflammatory response index = (NEU*MON)/LYM; AISI, aggregate inflammatory systemic index = (NEU*MON*PLT)/LYM; nSOFA, neonatal sequential organ failure assessment; ROC, receiver operating characteristic; AUC, the area under the ROC curve; OR, odds ratio; CIs, confidence intervals.

1. project basis

1.1 Research background

Necrotizing enterocolitis (NEC), a life-threatening gastrointestinal disorder predominantly, affects 5–12% of very low birth weight (VLBW) neonates, with 20–40% of cases progressing to severe disease requiring surgical intervention, while survivors often endure lifelong consequences, including short-bowel syndrome and neurodevelopmental impairments [1-4]. Despite advances in neonatal care, early identification of high-risk patients remains challenging due to the nonspecificity of clinical signs (e.g., abdominal distension, feeding intolerance) and radiographic findings (e.g., pneumatosis intestinalis), which frequently manifest only after irreversible bowel damage has occurred [5]. Furthermore, current diagnostic tools or conventional biomarkers lack both sensitivity and specificity for early risk stratification of NEC, underscoring an urgent need for biomarkers that integrate pathophysiological mechanisms to guide preemptive therapeutic strategies.

Emerging evidence underscores the multifactorial pathophysiology of NEC, where intestinal ischemia-reperfusion injury, exaggerated immune activation, and microbial dysbiosis converge to drive disease progression [6]. Hypoxic-ischemic injury constitutes a key pathological mechanism underlying intestinal mucosal damage in NEC, driving dysregulation of cellular processes including apoptosis, autophagy, proliferation, and migration within intestinal tissues [7]. In infants with NEC, persistent hypoxemia and compromised tissue perfusion induce metabolic reprogramming, marked by a shift toward glycolytic dominance—a compensatory adaptation to oxygen deprivation. Within this framework, serum lactate, as a well-established marker of tissue hypoperfusion and anaerobic metabolism, has garnered attention for its prognostic relevance. Elevated lactate levels correlate with transmural intestinal necrosis and predict fatal outcomes in NEC, reflecting the severity of ischemic insult [8,9]. However, lactate alone lacks specificity, as hyperlactatemia may arise from concurrent sepsis or perinatal asphyxia, limiting its standalone utility [10,11]. Recent studies propose combining lactate with

immune-inflammatory biomarkers (such as intestinal fatty acid binding protein or albumin) to address this limitation [12,13]. Peripheral blood cell analysis serves as a widely accessible and rapid auxiliary diagnostic tool in neonatal intensive care units (NICUs). Dynamic changes in peripheral white blood cell (WBC) counts and subset distribution have been validated as biomarkers closely associated with NEC [14]. Accumulating evidence underscores the involvement of lactate metabolism in immunomodulation and its influence on disease pathogenesis [15]. However, the combined utility of lactate levels and peripheral WBC profiles for NEC diagnosis and prognostic stratification remains unexplored.

As NEC progresses, particularly in cases of perforated NEC, it can evolve from a localized intestinal disease into a systemic illness characterized by a sepsis-like state and multi-organ dysfunction. The Neonatal Sequential Organ Failure Assessment (nSOFA) score is a validated tool designed to objectively quantify the degree of organ dysfunction in critically ill neonates by evaluating the respiratory, cardiovascular, and hematologic systems [16]. Studies have demonstrated that higher nSOFA scores are strongly and independently associated with mortality risk in neonates with respiratory distress syndrome, late-onset sepsis, and NEC [16-18]. However, single nSOFA lacks sufficient sensitivity for severe NEC subtypes like PNEC-specific risk stratification: while it predicts overall NEC mortality and the need for surgical intervention [16], existing studies have not specifically evaluated its performance in distinguishing NEC from non-perforated NEC or predicting PNEC-related mortality. The Lewis et al. [16] study included surgical NEC (e.g., laparotomy or peritoneal drain placement) but did not isolate PNEC as a distinct endpoint, leaving a critical gap in understanding how nSOFA performs in this highest-risk subgroup.

1.2 Creativity

To address these gaps, the present retrospective cohort study aims to explore the potential predictive value of integrating metabolic (lactate), hematologic (CBC), and organ dysfunction (nSOFA) metrics for NEC and its associated mortality in preterm infants. By combining these complementary markers, we aim to overcome the

limitations of single biomarkers and fill the knowledge gap regarding risk stratification for perforated NEC—an outcome that holds profound implications for clinical decision-making and improving the survival rate of preterm infants.

2. Research objective

This retrospective cohort study aims to enroll patients with necrotizing enterocolitis (NEC) from July 1, 2017 to December 31, 2022, conduct a comprehensive analysis of their clinical characteristics, metabolic indicators, laboratory parameters, prognosis, and outcomes, and identify indicators that may predict the progression of neonatal NEC and its associated mortality.

3. Research scheme and technical route

3.1 Research Design

The principal investigator (PI) and project leader of this study are Wei Zhou, chief physician of pediatrics of the Guangzhou Women and Children's Medical Center. In this study, a retrospective analysis was conducted on 220 enrolled neonates with necrotizing enterocolitis (NEC), with data collected from July 1, 2017 to December 31, 2022. Statistical methods including correlation analysis, multivariate logistic regression, mediation analysis, and receiver operating characteristic (ROC) curve analysis were employed to perform mutual analysis of the collected clinical data, laboratory parameters, and prognostic/mortality outcomes. This analysis aimed to identify meaningful biomarkers and establish a predictive model, thereby providing evidence for the pathophysiological mechanisms underlying NEC progression. It is anticipated that this will optimize clinical decision-making for NEC patients and improve the survival rate and quality of life of these neonates.

3.2 Sample size

220 newborns who suffered from necrotizing enterocolitis between July 1, 2017 to December 31, 2022.

3.3 data collection

Data collection was performed via structured extraction from electronic medical

records. The following variables were systematically retrieved: (1) Neonatal medical history, including sex, gestational age, birth weight, and history of birth asphyxia (e.g., Apgar scores); (2) Maternal medical history, particularly mode of delivery, chorioamnionitis, and premature rupture of membranes; (3) NEC-related clinical parameters: Bell's staging, clinical manifestations (abdominal distension, bloody stools, pneumoperitoneum), complications (patent ductus arteriosus [PDA], sepsis, shock), therapeutic interventions (mechanical ventilation, surgical treatment), and mortality; (4) CBC parameters, lactate levels, and parameters required for calculating the nSOFA score at the onset of NEC.

3.4 Inclusion criteria

(1) Diagnosis of NEC and having undergone surgical treatment, confirmed by clinical manifestations (e.g., abdominal distension, bloody stools), radiological evidence (pneumatosis intestinalis, portal venous gas, or pneumoperitoneum), and pathological examination in accordance with established guidelines;

(2) Availability of complete medical records for data extraction.

3.5 Excluded criteria

Data that meets any of the following conditions need to be eliminated:

(1) Congenital gastrointestinal malformations (e.g., intestinal atresia, Hirschsprung's disease) or spontaneous intestinal perforation;

(2) Hereditary metabolic disorders;

(3) Incomplete clinical data or refusal of participation.

3.6 Observation items

(1). Baseline Characteristics: Neonatal demographics (sex, gestational age, birth weight, small for gestational age [SGA] status), maternal factors (mode of delivery, premature rupture of membranes, chorioamnionitis), neonatal birth history (1-min Apgar score $\leqslant$ 7, intrauterine distress), and preterm complications (patent ductus arteriosus [PDA], respiratory distress syndrome [RDS], sepsis, shock), feeding pattern, and requirement for mechanical ventilation.

(2). Laboratory and Clinical Assessment Indicators: Hepatobiliary function:

Albumin, albumin/globulin ratio, total bile acid; Hematologic and inflammatory parameters: White blood cell (WBC), absolute neutrophil (NEU), lymphocyte (LYM), monocyte (MON), eosinophil (EOS) counts, red blood cell (RBC), platelet (PLT) count, mean platelet volume (MPV), platelet distribution width (PDW), C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), neutrophil-to-monocyte ratio (NMR), systemic inflammatory index (SII), systemic inflammatory response index (SIRI), aggregate inflammatory systemic index (AISI); Metabolic: Serum lactate, blood glucose; Organ dysfunction assessment: Neonatal Sequential Organ Failure Assessment (nSOFA) score.

(3). Primary Outcome Indicators:

- Occurrence of perforated necrotizing enterocolitis (PNEC);
- In-hospital mortality.

(4). Secondary Outcome Indicators:

- Bell's staging of NEC;
- surgical NEC

3.7 Statistical processing

Statistical analyses and graphic visualization were performed using SPSS 26.0 (IBM Corp.) and GraphPad Prism 9.0. Data for normally distributed, skewed distributed, and categorical variables were expressed as mean ± standard deviation, median (interquartile range), and frequency (%), respectively. Intergroup comparisons of categorical variables were conducted using the Chi-square (χ^2) test or Fisher's exact test, while continuous variables were analyzed using the Student's t-test or Mann-Whitney U test. Variables with $p < 0.05$ in univariate analysis were subsequently included in multivariate logistic regression analysis.

Mediation analysis was performed using the PROCESS macro 4.1, with 1000 bootstrap iterations to decompose the total effect into direct and indirect paths. Receiver operating characteristic (ROC) curve analysis was conducted using SPSS 26.0 to evaluate the predictive performance of individual and combined biomarkers. All analyses adopted two-tailed tests, and $p < 0.05$ was considered statistically

significant.

4. Ethics in clinical research

Clinical research will follow the World Medical Congress ' Helsinki Declaration ' and other relevant provisions. Before the study began, the clinical study was carried out after the ethics committee approved the test plan. Before each subject is selected for this study, the researcher has the responsibility to fully and comprehensively introduce the purpose, procedure and possible risks of this study to the subjects or their agents, and to sign a written informed consent form. The subjects should be informed that they have the right to withdraw from the study at any time. Informed consent should be retained as a clinical research document for review. The personal privacy and data confidentiality of the subjects will be protected during the study.

5. Research progress

July 2024 - August 2024: Project pre-planning, standardization of data collection, and training of personnel Training

August 2024 - March 2025: The specific implementation stage of the experiment, collecting case data

March 2025 - June 2025: Writing scientific research papers and project summary reports (1) Organizing research data and conducting statistical analysis; (2) Writing and submitting scientific research papers; (3) Summarizing research results and writing the project summary report.

6. Reference

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