

Predictive Value of Serum Total Calcium for Surgical Intervention in Neonatal Necrotizing Enterocolitis: A Single-Center Retrospective Case-Control Study

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Predictive Value of Serum Total Calcium for Surgical Intervention in Neonatal Necrotizing Enterocolitis: A Single-Center Retrospective Case-Control Study

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Background Serum calcium is a prognostic marker in severe infections, yet its association with surgical intervention in neonatal necrotizing enterocolitis (NEC) remains unclear. This study evaluated the independent predictive value of serum total calcium (TCa) measured within 24 h of disease onset for surgical treatment in NEC.

Methods This single-center retrospective case-control study enrolled 181 neonates with Bell's stage $\geq$ II NEC at the First Affiliated Hospital of Anhui Medical University (January 2016–June 2025), divided into conservative (n = 126) and surgical (n = 55) groups. Maternal, neonatal, and laboratory data collected within 24 h of NEC onset were retrieved. After univariate screening, variables were reduced by least absolute shrinkage and selection operator (LASSO) regression and entered into multivariate logistic regression to identify independent risk factors. Predictive efficacy was assessed by receiver operating characteristic (ROC) curve analysis, and correlations between TCa and inflammatory markers by Spearman rank analysis.

Results Of the 181 neonates, 55 (30.4%) underwent surgical treatment. The surgical group had significantly lower gestational age, birth weight, and TCa, and higher C-reactive protein (CRP) and procalcitonin (PCT) levels. Multivariate logistic regression identified elevated CRP (OR = 1.018, 95% CI 1.007–1.030; P = 0.003), elevated PCT

(OR = 1.014, 95% CI 1.002–1.027; P = 0.032), and decreased TCa (OR = 0.025, 95% CI 0.002–0.227; P = 0.002) as independent risk factors for surgical intervention. TCa showed higher predictive accuracy (Area under the Curve [AUC] = 0.733, 95% CI 0.653–0.813) than CRP (AUC = 0.665, 95% CI 0.574–0.756) and PCT (AUC = 0.718, 95% CI 0.636–0.801). TCa was weakly negatively correlated with CRP ($r_s = -0.198$, $P < 0.05$) and PCT ($r_s = -0.294$, $P < 0.001$).

Conclusions Serum TCa measured within 24 h of disease onset is significantly associated with surgical treatment in NEC and may serve as a valuable adjunctive indicator for evaluating surgical indications.

Keywords Necrotizing enterocolitis, Total calcium, Surgical intervention, Predictive value

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Introduction

Neonatal necrotizing enterocolitis (NEC) is a severe acute gastrointestinal disorder in neonates that frequently causes substantial impairment of intestinal function. Surgically treated neonates have a relatively high mortality rate[1], and survivors may develop complications including short bowel syndrome and intestinal stricture[2], while prolonged hospitalization imposes a considerable burden on the families of affected infants[3]. Therefore, timely surgical intervention for neonates with NEC who meet

surgical indications is of critical importance.

The absolute surgical indication for NEC is intestinal perforation, which may present as pneumoperitoneum on upright abdominal radiographs. However, among neonates with NEC-related intestinal perforation, pneumoperitoneum is preoperatively detected on imaging in fewer than 40% of cases[4], and the assessment of relative surgical indications remains challenging[5]. Clinically, by the time surgical treatment is required, the bowel is already partially necrotic, yet decisions regarding surgical timing and urgency may largely depend on the individual judgment of clinicians[6]. Therefore, it is essential to identify objective, reliable predictive indicators at the early stage of NEC in neonates requiring surgical intervention.

To date, laboratory markers with predictive value for NEC surgical indications include C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), and serum sodium (Na), among others[7–9]. However, any single indicator has limited predictive value for surgical indications; consequently, most studies[7–9] employ a combination of multiple indicators to predict the surgical risk of NEC. Identifying a single indicator with excellent predictive performance is of considerable importance for multi-indicator combined models in predicting NEC surgical indications.

Serum calcium levels typically decline during sepsis in adults, children, and neonates, and serve as an effective biomarker for assessing infection severity and predicting patient prognosis[10–12]. Dysregulated inflammatory response is a key pathogenic mechanism shared by sepsis and NEC. Experimental studies[13] have demonstrated that during NEC

pathogenesis, piezo-type mechanosensitive ion channel component 1 (Piezo1) protein mediates Ca^{2+} influx into the cytoplasm, suggesting that dysregulation of calcium homeostasis may be critically involved in the inflammatory cascade of NEC. Currently, few studies have reported the relationship between serum calcium levels and NEC, and the association between serum total calcium (TCa) levels and NEC surgical indications remains unclear. Therefore, this study aims to investigate the predictive value of TCa for NEC surgical indications.

Materials and Methods

Patients

This was a retrospective case-control study. The inclusion criteria were neonates admitted to the Neonatal Intensive Care Unit (NICU) of the First Affiliated Hospital of Anhui Medical University between January 2016 and June 2025, with a confirmed diagnosis of NEC and Bell stage $\geq$ II. The exclusion criteria were: (1) Bell stage I; (2) incomplete clinical data, including birth at other hospitals, surgery at other hospitals, or immediate transfer to another hospital after NEC onset; (3) congenital gastrointestinal malformations or inherited metabolic diseases. A total of 181 neonates met the eligibility criteria and were divided into two groups according to treatment modality: 55 in the surgical treatment group and 126 in the conservative treatment group. The patient selection process is shown in Fig. 1.

Data Collection

Clinical and laboratory data were extracted from the medical records of eligible neonates diagnosed with NEC (Bell stage $\geq$ II). For clinical variables, abdominal manifestations were recorded within 24 hours after NEC onset, whereas all other clinical data were collected from the period before NEC onset. Laboratory variables consisted of blood test results obtained within 24 hours after NEC onset and before surgical intervention.

Neonatal clinical variables included: gestational age (GA), birth weight (BW), sex(male), 1-minute Apgar score, 5-minute Apgar score, small for gestational age (SGA), intratracheal administration of pulmonary surfactant (PS), time to first enteral feeding, feeding method (exclusive breastfeeding, mixed feeding, formula feeding), postnatal age at NEC onset, use of intravenous antibiotics (UOA), red cell transfusion (RCT), intravenous immunoglobulin (IVIG) infusion, probiotic use, and concomitant sepsis. Abdominal manifestations included abdominal distension, vomiting, bloody stools, abdominal wall erythema, and abdominal mass.

Maternal clinical variables included: mode of delivery (cesarean section), antenatal intravenous corticosteroid administration (CA), intravenous magnesium sulfate administration, hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus (GDM), and premature rupture of membranes (PROM) > 18 hours.

Neonatal laboratory blood test indicators included: white blood cell count (WBC), platelet count (PLT), CRP, PCT, mean platelet volume (MPV), platelet distribution width (PDW), serum albumin (ALB), serum total calcium (TCa), serum sodium (Na), and serum potassium (K).

All clinical data described above were extracted from the electronic medical record system of the Medical Records Department at the First Affiliated Hospital of Anhui Medical University.

Definitions of clinical conditions

NEC was diagnosed according to the Bell staging system[14] (Stage I: suspected; Stage II: definite; Stage III: advanced). The main clinical manifestations at onset included vomiting, abdominal distension, and bloody stools, among others, and the primary radiographic findings included pneumatosis intestinalis and portal venous gas, among others. Surgical indications were defined as intestinal perforation (pneumoperitoneum) or persistent clinical deterioration during conservative treatment[15, 16] (including refractory abdominal distension, abdominal wall erythema, and progressively worsening signs of peritonitis). All surgeries were performed via exploratory laparotomy.

SGA was defined as birth weight below the 10th percentile for gestational age. Sepsis was defined as a positive blood culture or clinically diagnosed sepsis with a negative blood culture. The threshold for red blood cell transfusion was determined primarily by hemoglobin/hematocrit levels, in conjunction with the neonate's clinical condition and postnatal age. Time to first feeding was defined as the postnatal age at initiation of breast milk or formula feeding. Antenatal corticosteroid administration was defined as maternal dexamethasone treatment before delivery. TCa was defined as the lowest value measured within 24 hours after NEC onset.

Statistical Analysis

Data analysis was performed using R software (version 4.5.3). Normally distributed variables were expressed as the mean $\pm$ standard deviation(SD), and between-group comparisons were performed using the independent-samples t-test; non-normally distributed variables were expressed as median (interquartile range), and between-group comparisons were performed using the Wilcoxon rank-sum test. Categorical variables were presented as frequencies (percentages), and between-group comparisons were performed using the chi-square (χ^2) test or Fisher's exact test. To avoid omitting variables of potential importance, variables with a P-value < 0.2 in univariate analysis were entered into least absolute shrinkage and selection operator (LASSO) regression to screen for potential predictors, followed by multivariate logistic regression analysis to identify independent risk factors for surgical treatment of NEC. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive value of individual variables for NEC surgical indications. Spearman rank correlation analysis was used to assess the correlations among the risk factors. A P-value < 0.05 was considered statistically significant.

Results

Baseline characteristics and univariate analysis of neonatal clinical variables

Between January 2016 and June 2025, a total of 308 neonates were diagnosed with NEC.

Twenty-two cases with incomplete clinical data were excluded, including 12 born at other hospitals, 9 who underwent surgery at other hospitals, and 1 immediately transferred to another hospital after NEC onset at the parents' request. In addition, 102 neonates with Bell stage I NEC, 1 neonate with Hirschsprung's disease, and 2 neonates with congenital intestinal malrotation were excluded. The remaining 181 neonates with Bell stage $\geq$ II NEC were enrolled and divided into a surgical group (n = 55) and a conservative treatment group (n = 126) according to treatment modality (Fig. 1). Among the 181 enrolled neonates, 174 (96.1%) were preterm and 7 (3.9%) were term. In the conservative treatment group, 121 (96.0%) were preterm and 5 (4.0%) were term. In the surgical group, 53 (96.4%) were preterm and 2 (3.6%) were term (Fig. 2).

Compared with the conservative treatment group, neonates in the surgical group had significantly lower gestational age [30.57 (28.79–32.50) weeks vs 32.00 (30.43–33.57) weeks] and birth weight [1.28 (1.14–1.54) kg vs 1.57 (1.35–1.87) kg], and both differences were statistically significant ($P < 0.001$). See Table 1 and Fig. 3.

Compared with the conservative treatment group, the surgical group had significantly higher rates of intratracheal PS administration (47.3%), sepsis (36.4%), red blood cell transfusion (16.4%), use of intravenous antibiotics (85.5%), and abdominal distension (98.2%), with all differences statistically significant (all $P < 0.05$). No significant between-group differences were found in SGA, sex, 1-minute and 5-minute Apgar scores, postnatal age at NEC onset, time to first feeding, feeding method (exclusive breastfeeding, mixed feeding, formula feeding), the use of intravenous immunoglobulin (IVIG), or the

incidence of vomiting, bloody stools, abdominal wall erythema, and abdominal mass (all $P > 0.05$). See Table 1 and Fig. 3.

Table 1 Comparison of neonatal and maternal clinical characteristics between the conservative treatment group and the surgical treatment group

Variables	Conservative NEC(126)	Surgical NEC(55)	<i>P</i> value
Gestational age(weeks)	32.00(30.43–33.57)	30.57(28.79–32.50)	<0.001
Birth weight(kg)	1.57(1.35–1.87)	1.28(1.14–1.54)	<0.001
SGA	37(29.4)	13(23.6)	0.428
Male	82(65.1)	35(63.6)	0.852
1-min Apgar score	8(6–10)	8(6–9)	0.454
5-min Apgar score	9(8–10)	9 (8–10)	0.449
Age at onset(days)	14 (9–21)	15(10.5–23.5)	0.487
Intratracheal PS administration	36(28.6)	26(47.3)	0.017
Use of intravenous antibiotics	88(69.8)	47(85.5)	0.027
Probiotic use	57(45.2)	23(41.8)	0.670
Sepsis	26(20.6)	20(36.4)	0.031
Time to first enteral feeding(days)	1.34(0.84–2.49)	1.33 (0.88–1.96)	0.645
Feeding methods			0.505
Exclusive breastfeeding	4(3.2)	2(3.6)	
Mixed feeding	28(22.2)	8(14.5)	
Formula feeding	94(74.6)	45(81.8)	
Red cell transfusion	8(6.3)	9(16.4)	0.034
IVIG infusion	14(11.1)	7(12.7)	0.755
Abdominal manifestations			
Abdominal distension	109(86.5)	54(98.2)	0.016
Vomiting	28(22.2)	13(23.6)	0.834
Bloody stools	45(35.7)	12(21.8)	0.064
Abdominal wall erythema	0(0)	2(3.6)	0.091
Abdominal mass	30(23.8)	10(18.2)	0.401
Maternal clinical variables			
HDP	32(25.4)	12(21.8)	0.606
Cesarean section	88(69.8)	42(76.4)	0.370
GDM	21(16.7)	15(27.3)	0.100
PROM>18hs	35(27.8)	15(27.3)	0.944
Corticosteroid use	98(77.8)	45(81.8)	0.678
Magnesium sulfate use	79(62.7)	42(76.4)	0.073

Notes: All values are presented as the mean± SD or n (%) or as the median (interquartile range).

Abbreviations: NEC, necrotizing enterocolitis; SGA, small for gestational age; PS, pulmonary surfactant; IVIG, intravenous immunoglobulin; HDP, hypertensive disorders of pregnancy; GDM, gestational diabetes mellitus; PROM, premature rupture of membranes.

Table 2 Comparison of neonatal laboratory indicators between the conservative and surgical treatment groups

Variables	Conservative NEC(126)	Surgical NEC(55)	<i>P</i> value
WBC	6.28(4.21–9.96)	5.88(3.50–8.99)	0.293
PLT	250.30 ±112.39	256.21±100.90	0.727
CRP	21.65(7.11–40.64)	39.71(17.73–72.03)	<0.001
PCT	3.16(0.92–9.13)	19.66(3.32–36.45)	<0.001
MPV	11.60(10.93–12.40)	11.90(11.30–12.65)	0.027
PDW	14.50(12.53–16.58)	15.30(13.05–17.95)	0.130
Albumin	29.20(26.35–31.78)	27.80(24.00–31.85)	0.090
Na	136.75(134.53–139.20)	132.90(130.50–135.30)	<0.001
K	4.71(4.40–5.09)	4.93(4.53–5.36)	<0.05
TCa	2.39(2.30–2.48)	2.26(2.16–2.36)	<0.001

Notes: All values are presented as the mean± SD or n (%) or as the median (interquartile range).

Abbreviations: NEC, necrotizing enterocolitis; WBC, white blood cell count; PLT, platelet count; CRP, C-reactive protein; PCT, procalcitonin; MPV, mean platelet volume; PDW, platelet distribution width; Na, sodium; K, potassium; TCa, total calcium.

Univariate Analysis of Neonatal Laboratory Indicators

Compared with the conservative treatment group, neonates in the surgical group had significantly higher levels of CRP [39.71 (17.73–72.03) mg/L vs. 21.65 (7.11–40.64) mg/L], PCT [19.66 (3.32–36.45) ng/mL vs. 3.16 (0.92–9.13) ng/mL], MPV [11.90 (11.30–12.65) vs. 11.60 (10.93–12.40)], and K [4.93 (4.53–5.36) mmol/L vs. 4.71 (4.40–5.09) mmol/L] within 24 hours after NEC onset, with all differences statistically significant (all *P* < 0.05). The surgical group also had significantly lower TCa [2.26 (2.16–2.36) mmol/L vs. 2.39 (2.30–2.48) mmol/L] and Na [132.90 (130.50–135.30) mmol/L vs. 136.75 (134.53–139.20) mmol/L] levels (both *P* < 0.001). No significant between-group differences were observed in WBC, PLT, PDW, or ALB levels (all *P* > 0.05). See Table 2

and Fig. 3.

Univariate Analysis of Maternal Clinical Variables

Compared with the conservative treatment group, no significant between-group differences were found in the rates of cesarean delivery, GDM, HDP, PROM > 18 hours, antenatal corticosteroid administration, or magnesium sulfate use among mothers (all $P > 0.05$). See Table 1.

Table 3 Risk factors for surgical treatment of NEC identified by multivariate logistic regression analysis

Variables	β	SE	z-value	OR	(95% CI)	<i>P</i> value
PCT	0.014	0.006	2.147	1.014	1.002–1.027	0.032
TCa	−3.702	1.185	−3.124	0.025	0.002–0.227	0.002
CRP	0.018	0.006	2.992	1.018	1.007–1.030	0.003

Notes: NEC, necrotizing enterocolitis; β , regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

LASSO Regression and Multivariate Logistic Regression

Thirteen variables showed statistically significant differences ($P < 0.05$) in univariate analysis. To avoid omitting variables of potential importance (eg, albumin), 19 variables with $P < 0.2$ in univariate analysis were entered into LASSO regression, including GA, BW, intratracheal PS administration, sepsis, PCT, MPV, K, Na, TCa, PDW, CRP, albumin, GDM, intravenous antibiotic use, red cell transfusion, magnesium sulfate, abdominal distension, bloody stools, and abdominal wall erythema. Ten-fold cross-validation was performed, and the optimal penalty parameter was determined using the one-standard-error rule ($\lambda = \lambda_{1se}$). LASSO regression ultimately retained three variables with non-zero

coefficients: CRP, PCT, and TCa (Fig. 4 and 5). Multivariate logistic regression analysis including these three variables demonstrated that elevated CRP (OR = 1.018, 95% CI: 1.007–1.030, P = 0.003), elevated PCT (OR = 1.014, 95% CI: 1.002–1.027, P = 0.032), and decreased TCa (OR = 0.025, 95% CI: 0.002–0.227, P = 0.002) within 24 hours after NEC onset were independent risk factors for surgical treatment of NEC. See Table 3.

Table 4 ROC curve analysis results of CRP, PCT, and TCa for predicting surgical treatment of NEC

Variables	AUC (95% CI)	Cut-off value	Sensitivity	Specificity	Youden_index
PCT	0.718(0.636–0.801)	6.350	69.1%	70.6%	1.397
TCa	0.733(0.653–0.813)	2.295	61.8%	76.2%	1.380
CRP	0.665(0.574–0.756)	53.145	43.6%	88.1%	1.317

Abbreviations: NEC, necrotizing enterocolitis; ROC, receiver operating characteristic; AUC, under the curve; CI, confidence interval.

ROC Curve Analysis of Individual Predictive Indicators

ROC curve analysis was performed separately for the three independent risk factors identified by multivariate logistic regression (elevated CRP, elevated PCT, and decreased TCa) to evaluate the predictive value of each indicator for surgical risk in NEC. The AUCs of CRP and PCT were 0.665 (95% CI: 0.574–0.756) and 0.718 (95% CI: 0.636–0.801), respectively, both lower than that of TCa (AUC = 0.733, 95% CI: 0.653–0.813). See Table 4 and Fig. 6.

Spearman Rank Correlation Analysis

Given that CRP, PCT, and TCa were not normally distributed, the Spearman rank correlation coefficient was used to assess the correlation between serum total calcium

(TCa) and inflammatory markers (CRP and PCT) in all 181 neonates with NEC (surgical and conservative groups combined). TCa was significantly negatively correlated with CRP ($r_s = -0.198$, $P < 0.05$) and with PCT ($r_s = -0.294$, $P < 0.001$). These results indicate that TCa tends to decrease with increasing CRP and PCT levels; however, the absolute values of both correlation coefficients were less than 0.3, indicating a weak association. Comparatively, the correlation between TCa and PCT was slightly stronger than that with CRP. See Fig. 7.

Discussion

NEC is an acute necrotizing intestinal disease in neonates. Neonates who progress to intestinal perforation and necrosis require timely surgical intervention; however, effective predictive indicators for the relative surgical indications of NEC remain lacking. Therefore, identifying indicators with predictive value for surgical treatment of NEC is of particular importance. The present study found that elevated CRP and PCT, as well as decreased TCa, were independent risk factors for the need for surgical treatment in neonates with NEC. To the best of our knowledge, this is the first clinical study to report an association between TCa and the need for surgical treatment in NEC, and the predictive value of TCa for surgical treatment was higher than that of CRP and PCT.

As inflammatory markers, CRP and PCT are widely used in the clinical diagnosis of sepsis. During NEC pathogenesis, abundant inflammatory cytokines and chemokines are produced, leading to disruption of intestinal mucosal integrity[17]. As a routine inflammatory marker, CRP reflects the systemic inflammatory response and can predict

disease severity[18] and the need for surgical intervention[8, 9, 19] in NEC. Tang et al[9] found that a surgical prediction model for NEC incorporating six indicators, including CRP, serum lactate, white blood cell count, and absolute lymphocyte count, achieved an AUC as high as 0.946, demonstrating good predictive performance. A meta-analysis[8] including 18 studies with a total of 1,104 surgically treated and 1,686 conservatively treated neonates showed that CRP remains an independent risk factor for surgical treatment of NEC, consistent with the findings of the present study.

Compared with CRP, PCT has higher specificity for bacterial infection and begins to rise 3–4 hours after infection[20]. During NEC pathogenesis, bacteria can breach innate immune defenses and invade the intestinal epithelium, thereby triggering inflammation and tissue necrosis[21]. The present study showed that the predictive value of PCT for NEC surgery (AUC = 0.718) was superior to that of CRP (AUC = 0.665), consistent in trend with a previous study by Yu et al[22], who also reported higher predictive performance of PCT (AUC = 0.864) than CRP (AUC = 0.783) for surgical treatment of NEC. In addition, another study[23] has indicated that PCT represents a risk factor for intestinal stricture following the onset of NEC, and intestinal stricture may increase the likelihood of requiring surgical intervention. Furthermore, a preoperative prediction model combining PCT, bloody stools, fever, and shock showed increased mortality in neonates undergoing surgical treatment for NEC[24], suggesting that elevated PCT may reflect more severe inflammation and a greater degree of intestinal necrosis.

Serum total calcium exists in three forms: ionized calcium (iCa), protein-bound

calcium (primarily bound to albumin), and anion-bound calcium. Blood calcium plays important roles in muscle contraction, blood coagulation, enzyme activity, neural excitability, hormone secretion, and cell adhesion[25]. The association between serum calcium levels and sepsis has been investigated in adults[11], children[12], and neonates[10, 26]. Inflammation is a key pathogenic mechanism shared by sepsis and NEC, and experimental studies[13] have demonstrated that activation of Piezo1 protein mediates Ca^{2+} influx into the cytoplasm in an in vitro NEC intestinal epithelial model. We therefore hypothesized that serum calcium levels may be associated with NEC; however, no studies to date have examined the association between TCa and surgical treatment of NEC. The present study found that decreased TCa was an independent risk factor for surgical treatment of NEC.

The present study found that, among 181 NEC neonates, CRP was weakly negatively correlated with TCa ($r_s = -0.198$), whereas PCT showed a slightly stronger negative correlation with TCa ($r_s = -0.294$), suggesting that higher PCT levels are associated with lower TCa levels and, in neonates with NEC, a greater risk of requiring surgery. A study of sepsis in an elderly population (aged > 60 years)[11] showed that PCT was also negatively correlated with TCa ($r_s = -0.2957$), consistent in trend with the present study; moreover, TCa has value in the early identification of sepsis and assessment of disease severity[11]. Decreased iCa has been associated with increased 28-day mortality after admission in pediatric sepsis[12], and reduced iCa has also been linked to increased mortality in neonatal sepsis[26]. In very low birth weight infants, decreased iCa is not

only associated with sepsis-related mortality but can also predict adverse outcomes, including periventricular leukomalacia (PVL), grade 3–4 intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP) stage ≥ 3 , and stage III NEC[10]. It should be noted that the absolute values of correlation coefficients between TCa and inflammatory markers were relatively small, indicating that the reduction in serum calcium cannot be fully explained by the inflammatory burden. After adjusting for albumin, TCa remained an independent predictor for surgical intervention in NEC. Limited by the retrospective design, calcium-regulating hormones were not collected in this study. Calcium homeostasis disruption may be affected by these unmeasured variables as well as other metabolic disturbances. These findings further suggest that TCa carries independent value for assessing surgical risk, distinct from conventional inflammatory markers.

As this is a retrospective clinical observation, the exact mechanism underlying reduced serum TCa levels in the surgical group remains unclear, and relevant mechanistic basic data are limited. Based on the available published literature[13, 27, 28], we speculate that calcium-signalling pathways may be involved in NEC-associated intestinal injury and contribute to the decline in circulating TCa levels. The detailed mechanistic hypotheses are described below.

Piezo1 is a mechanosensitive ion channel widely expressed in intestinal epithelial cells (IECs)[29, 30]. It senses mechanical crowding and stretch[31] and transduces mechanical forces into intracellular signaling responses. Upon activation, Piezo1 mediates Ca^{2+} influx into intestinal stem cells, regulating the Notch/Wnt signaling pathway and cell

differentiation[32]. Following activation, Piezo1 promotes Ca^{2+} uptake into the endoplasmic reticulum (ER) via the ER Ca^{2+} -ATPase pump, and subsequently triggers Ca^{2+} release from the ER into the cytosol through inositol trisphosphate receptor 2 (IP3R2)[33], leading to increased cytosolic Ca^{2+} concentration and ER Ca^{2+} depletion, thereby activating the ER stress response and inducing apoptosis[34, 35]. An in vitro NEC model using the human colorectal adenocarcinoma cell line Caco-2 confirmed that Piezo1-mediated Ca^{2+} influx triggers ER stress and apoptosis, and that chelation of cytosolic calcium effectively attenuates Piezo1-induced ER stress and intestinal epithelial cell apoptosis[27]. Intraoperative findings in neonates with NEC requiring surgical treatment have demonstrated varying degrees of intestinal necrosis and even perforation. We therefore speculate that Ca^{2+} influx-triggered ER stress and apoptosis may represent one mechanism underlying the decreased serum TCa observed in the surgical group of the present study.

The Ca^{2+} /calmodulin-dependent protein kinase (CaMK) family mainly includes CaMKI, CaMKII, and CaMKIV, among others. These kinases are activated primarily by elevated intracellular Ca^{2+} concentrations and participate in regulating the expression of downstream target genes[36]. Clinical studies[37] have shown that serum levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) are elevated in neonates after NEC onset compared with controls. Piezo1 protein is expressed in intestinal epithelial cells of both neonates and mice, with higher expression in preterm infants born at < 34 weeks of gestation[13]. In vitro NEC model experiments have demonstrated that Piezo1-mediated

elevation of cytosolic Ca^{2+} activates CaMKII, promoting the interaction between CaMKII and nuclear factor- κB (NF- κB) and subsequent NF- κB activation. This leads to significantly increased expression of proinflammatory cytokines TNF- α and IL-6 in mouse serum and intestinal tissue, exacerbating intestinal inflammation and cell death. Conversely, Piezo1 knockout in both in vivo and in vitro NEC models attenuates NEC-associated inflammation[13]. We therefore speculate that Ca^{2+} influx activates CaMKII-dependent signaling pathways to exacerbate NEC inflammation, representing another mechanism underlying the decreased TCa observed in the surgical group.

Previous studies have shown that the release of interleukin-17 (IL-17) disrupts tight junctions in intestinal epithelial cells during NEC pathogenesis[38]. A study by Alganabi et al[28] confirmed that CaMKIV expression is localized to the mouse villous epithelium. In a mouse model of NEC, the NEC group showed significantly elevated expression of CaMKIV and its downstream target genes, cAMP-responsive element modulator (CREM) and IL-17, compared with normal controls; IL-6 expression was also higher in the NEC group, and these mice exhibited severe intestinal damage. However, Alganabi et al[28] did not investigate whether Ca^{2+} influx occurs in villous epithelial cells during NEC pathogenesis. Given that previous studies[13, 27] have demonstrated that Ca^{2+} enters intestinal epithelial cells via Piezo1-mediated influx during NEC, we speculate that Ca^{2+} influx into the cytoplasm of villous epithelial cells activates the CaMKIV signaling pathway, leading to severe intestinal damage, which may partially account for the decreased TCa observed in the surgical group.

Toll-like receptor 4 (TLR4) is one of the key pathways in NEC pathogenesis. It recognizes lipopolysaccharide (LPS), a component of Gram-negative bacteria[39]. Myeloid differentiation protein 2 (MD2), a critical accessory component of the TLR4 signaling pathway, is significantly elevated in intestinal tissue during the acute phase of NEC[40], and the MD2 inhibitor (MI) attenuates inflammation and intestinal mucosal injury by blocking the TLR4-MD2/NF- κ B signaling axis[40]. Upregulation of TLR4 expression induces ER stress in intestinal stem cells, leading to apoptosis in the intestinal crypt region[41]. Of note, in addition to mechanical stimuli, Piezo1 channel activation may also be regulated by bacterium-derived inflammatory signals. To date, no studies have directly verified the interaction between TLR4 and Piezo1 in NEC-derived intestinal epithelial cells. Nevertheless, experiments in other cell systems have demonstrated that LPS-triggered TLR4 activation facilitates Piezo1-dependent calcium influx. In the human pancreatic neuroendocrine tumor cell line BON-1, LPS stimulation increases TLR4 expression, and TLR4-Piezo1 binding enhances Ca^{2+} influx in BON-1 cells, leading to increased 5-HT secretion[42]. Geng et al[43] similarly found that LPS stimulation or *Escherichia coli* infection promotes the binding of TLR4 to Piezo1 in macrophages, thereby inducing Ca^{2+} influx. Conversely, TLR4 knockout inhibits the elevation of intracellular Ca^{2+} in neurons induced by oxygen-glucose deprivation (OGD) or LPS treatment[44]. Given the pivotal role of TLR4 in intestinal injury during NEC, we hypothesise that the TLR4-Piezo1 interaction may serve as an auxiliary mechanism contributing to the pathological process of NEC and partially explaining the reduced

circulating TCa levels in neonates requiring surgery. This hypothesis remains to be validated by cell-specific experiments targeting intestinal epithelium.

In summary, based on available pre-clinical evidence, we propose the following scientific hypothesis: under NEC conditions, inflammatory and mechanical stimuli activate the mechanosensitive channel Piezo1 (an effect potentially augmented by the TLR4-Piezo1 interaction), which mediates extracellular Ca^{2+} influx into intestinal epithelial cells. Subsequent intracellular calcium overload amplifies intestinal injury and systemic inflammatory responses by triggering endoplasmic reticulum stress and apoptosis, as well as activating downstream cascades including CaMKII-NF- κ B and CaMKIV-IL-17, ultimately leading to decreased circulating TCa levels. It should be emphasized that, as a retrospective clinical study, our work can only demonstrate a correlation between TCa and the requirement for surgical intervention in NEC. These causal regulatory relationships remain to be validated in future in-vitro and in-vivo experiments.

This retrospective study has several limitations. First, iCa is the biologically active form of calcium in vivo, and studies[13, 27] have confirmed that Piezo1-mediated Ca^{2+} influx is involved in NEC pathogenesis. We speculate that the decrease in serum TCa is primarily attributable to a reduction in the iCa fraction; however, iCa data were missing for a substantial proportion of cases, so TCa was selected as the predictive variable. The use of iCa, which is more biologically representative, may yield better predictive performance for NEC surgical risk. Second, owing to missing abdominal radiographs in

some cases and the subjective nature of radiographic interpretation, imaging findings after disease onset (eg, pneumatosis intestinalis) were not incorporated into this study, which constitutes a limitation. Third, as a single-center study with a relatively small sample size and no external validation, the generalizability of our conclusions should be interpreted with caution. Fourth, the predictive performance of any single indicator is limited, and a multi-variable combined model may achieve better performance in predicting NEC surgical risk. Our team aims to expand the case cohort in subsequent studies to develop a multivariable surgical-risk prediction model for NEC incorporating TCa, CRP and PCT, followed by external validation. Meanwhile, basic experimental studies will be performed to further verify the role of calcium-signalling pathways in the pathogenesis of NEC and the evaluation of surgical risk.

Conclusion

The present study found that elevated CRP and PCT, as well as decreased TCa, were independent risk factors for the need for surgical treatment in neonates with NEC, and TCa exhibited superior predictive performance for surgical treatment compared with CRP and PCT. Therefore, as a cost-effective, convenient, and readily available laboratory indicator, TCa may serve as an adjunctive reference for the clinical assessment of surgical indications in NEC.

Abbreviations

AUC	Area under the curve
BW	Birth weight
CaMK	Ca ²⁺ /calmodulin-dependent protein kinase

439	CI	Confidence interval
440	CRP	C-reactive protein
441	GA	Gestational age
442	GDM	Gestational diabetes mellitus
443	HDP	Hypertensive disorders of pregnancy
444	iCa	Ionized calcium
445	IVIG	Intravenous immunoglobulin
446	K	Potassium
447	LASSO	Least absolute shrinkage and selection operator
448	MPV	Mean platelet volume
449	Na	Sodium
450	NEC	Necrotizing enterocolitis
451	OR	Odds ratio
452	PCT	Procalcitonin
453	PDW	Platelet distribution width
454	Piezo1	Piezo-type mechanosensitive ion channel component 1
455	PLT	Platelet count
456	PROM	Premature rupture of membranes
457	PS	Pulmonary surfactant
458	ROC	Receiver operating characteristic
459	SE	Standard error
460	SGA	Small for gestational age
461	TCa	Total calcium
462	WBC	White blood cell count

463

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468

469 **Author contributions**

470 Zhixuan Yuan, Yang Wang, and Shenggang Ding designed the study. Yang Wang and
471 Shenggang Ding provided administrative support. Zhixuan Yuan and Xuejie Zheng
472 collected and curated the data. Zhixuan Yuan analyzed the data and drafted the manuscript.
473 Ming Chen critically revised the manuscript. All authors reviewed and approved the final
474 manuscript.

475

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478

479 **Data availability**

480 Data from this study are available from the corresponding author upon request.

481

Declarations

Ethical Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Approval No. KY2026-10-09). Owing to the retrospective nature of the study and the de-identification of patient data, the requirement for signed informed consent from the parents or legal guardians was waived with the approval of the Ethics Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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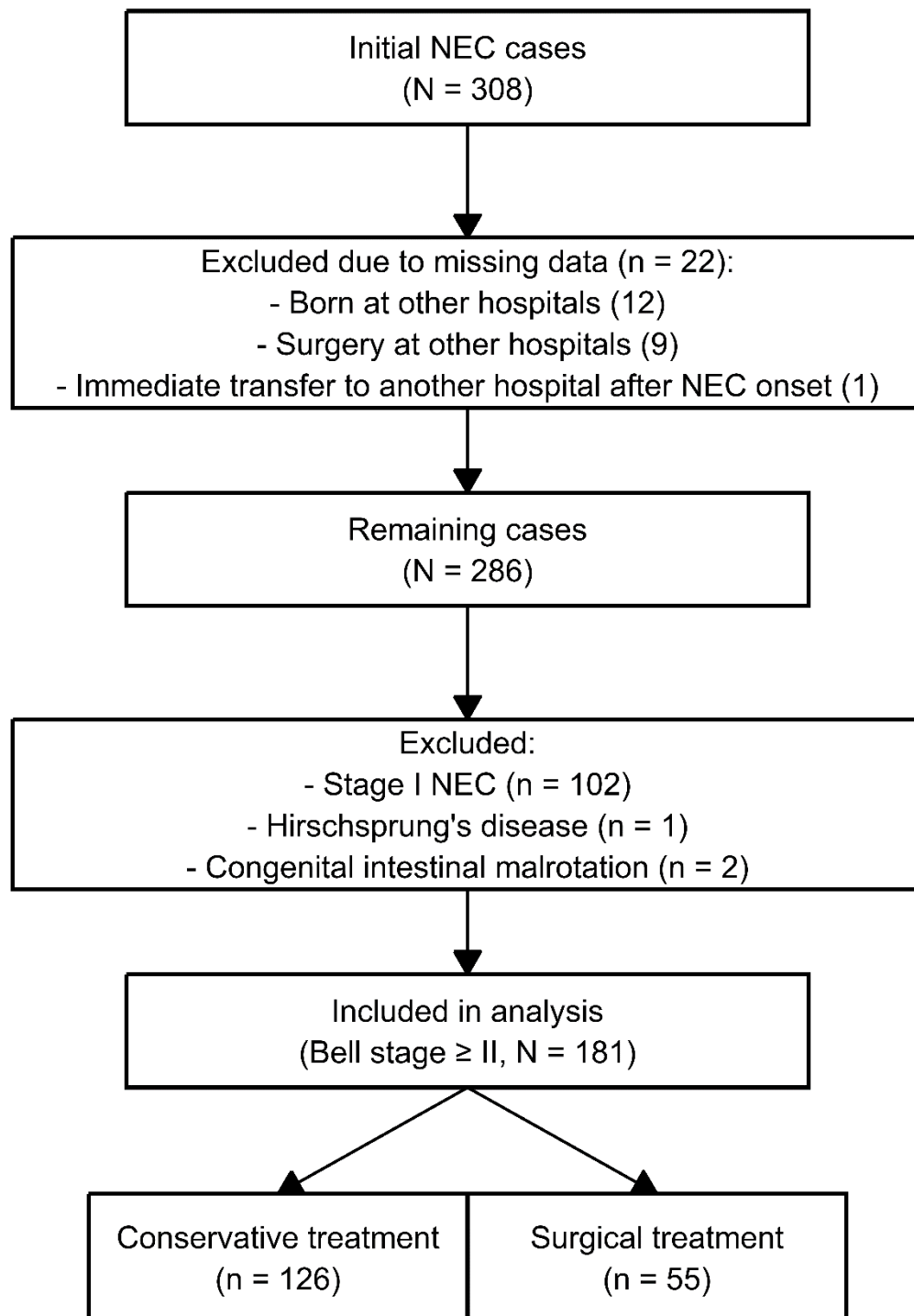


Fig. 1 Flow chart of this study.

Abbreviations: NEC, necrotizing enterocolitis.

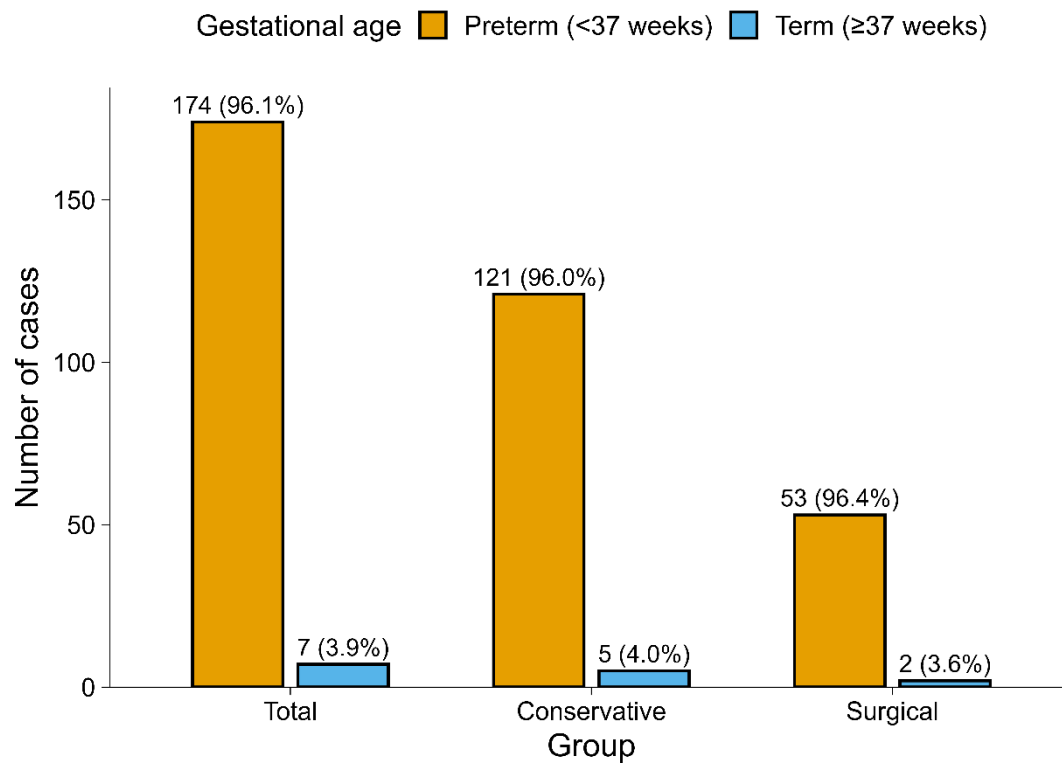


Fig. 2 Distribution of preterm and term neonates with NEC in the total cohort (n = 181), conservative group (n = 126), and surgical group (n = 55).

Abbreviations: NEC, necrotizing enterocolitis.

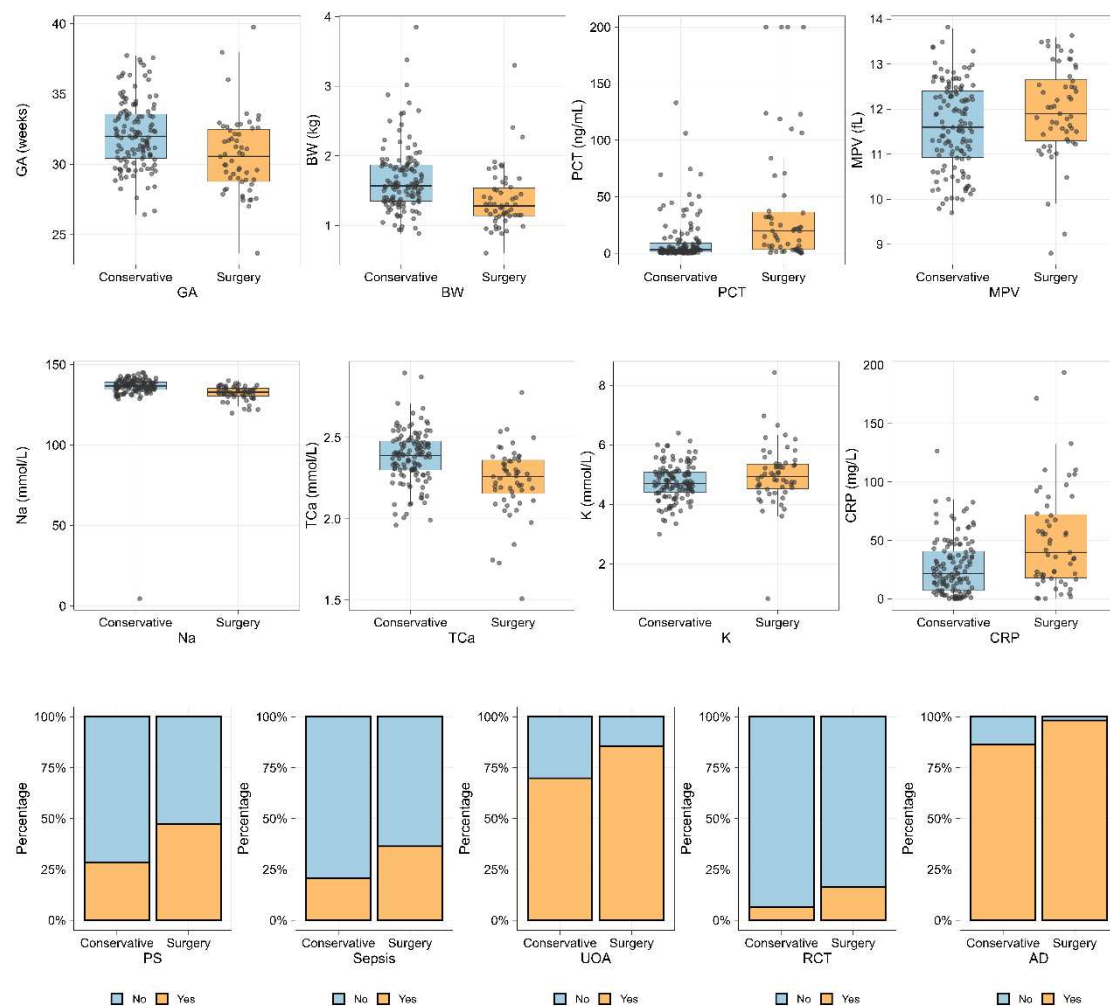


Fig. 3 Variables with statistically significant differences ($P < 0.05$) in univariate analysis between the surgical and conservative groups of NEC.

Abbreviations: NEC, necrotizing enterocolitis; GA, gestational age; BW, birth weight; PCT, procalcitonin; MPV, mean platelet volume; Na, sodium; TCa, total calcium; K, potassium; CRP, C-reactive protein; PS, pulmonary surfactant; UOA, use of intravenous antibiotics; RCT, red cell transfusion; AD, abdominal distension.

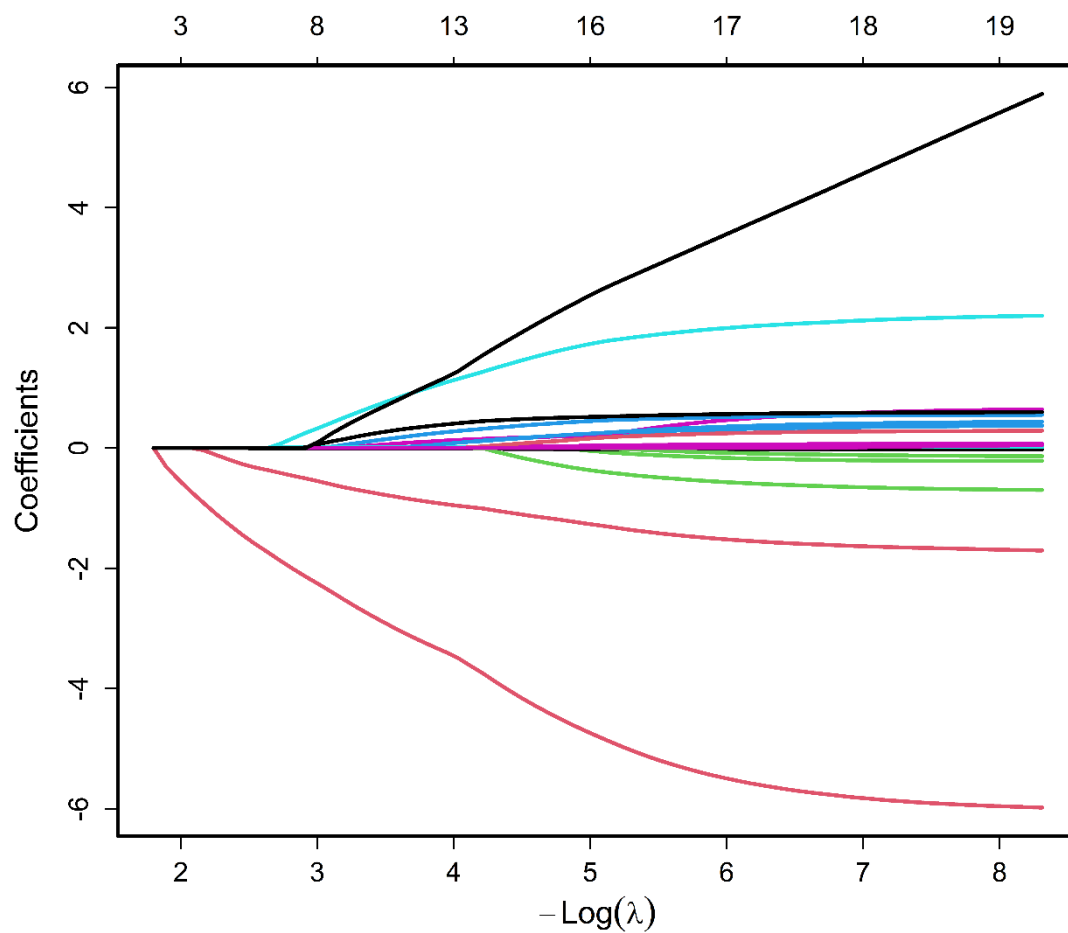


Fig. 4 Coefficient profile plot of LASSO regression.

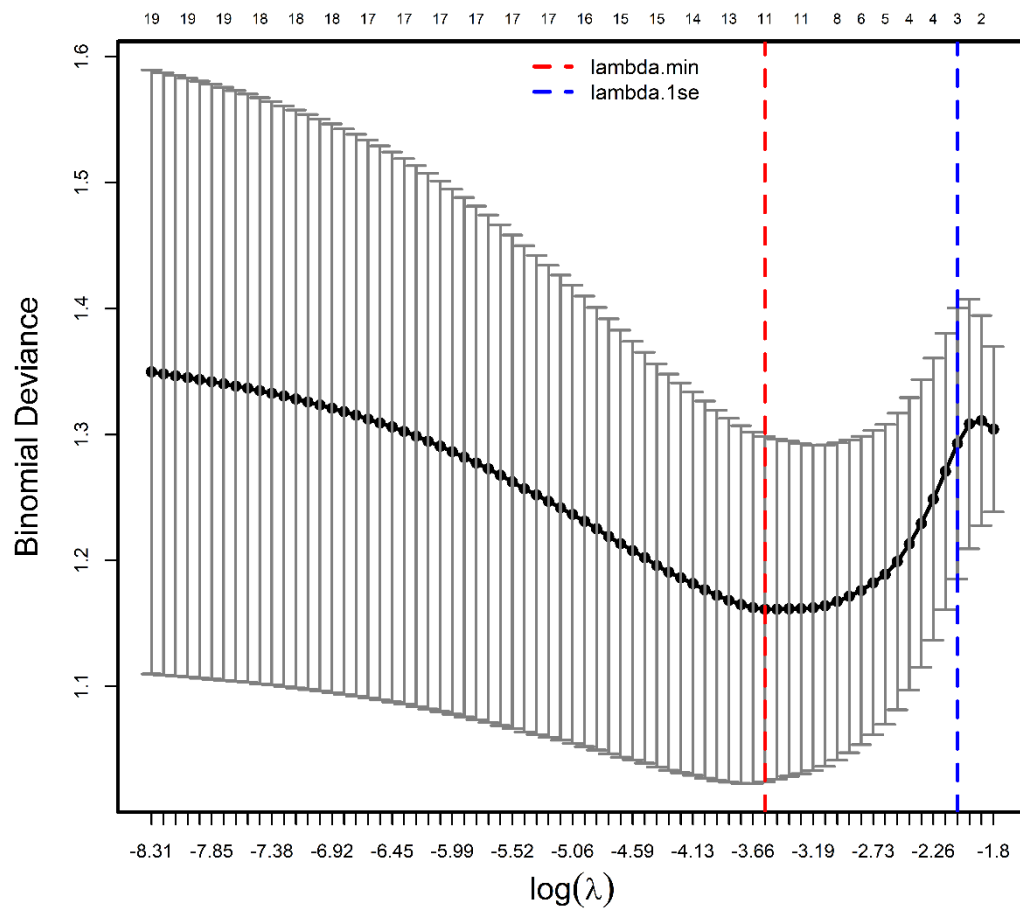


Fig. 5 Cross-validation curve for LASSO regression.

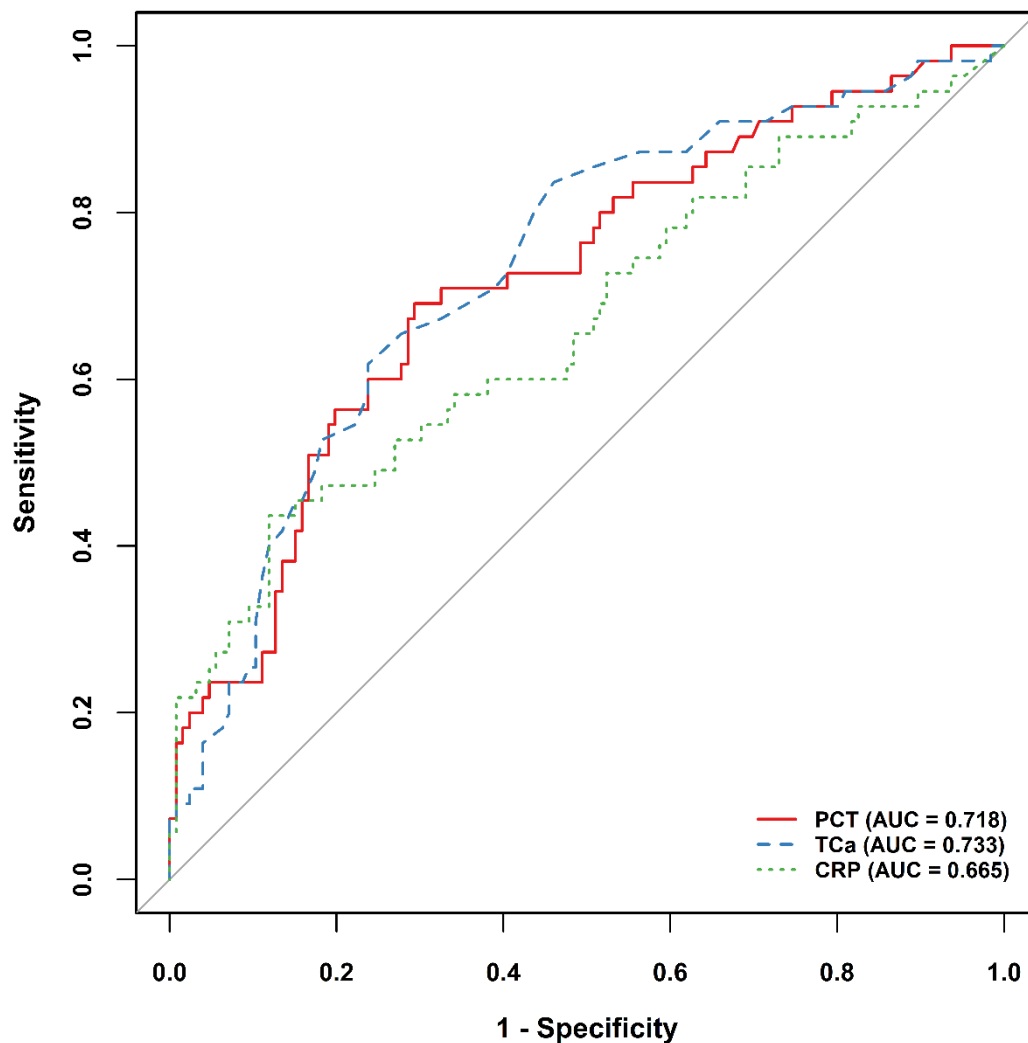


Fig. 6 ROC curve analysis of the predictive value of CRP, PCT, and TCa for surgical treatment of NEC.

Abbreviations: NEC, necrotizing enterocolitis; ROC, receiver operating characteristic; PCT, procalcitonin; TCa, total calcium; CRP, C-reactive protein.

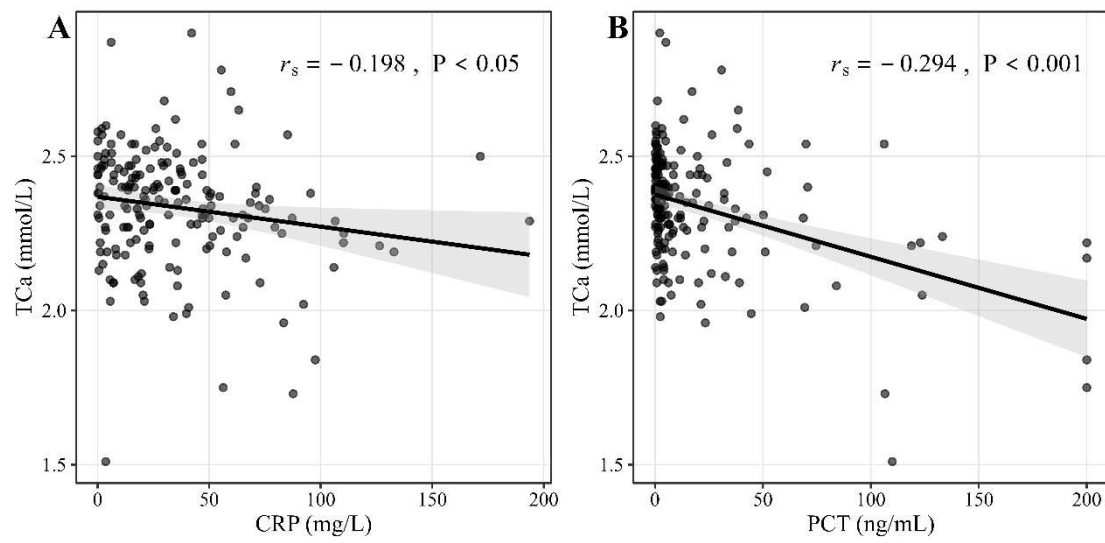


Fig. 7 (A) Spearman correlation between TCa and CRP in neonates with NEC; (B) Spearman correlation between TCa and PCT in neonates with NEC.
Abbreviations: NEC, necrotizing enterocolitis; TCa, total calcium; CRP, C-reactive protein; PCT, procalcitonin.