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Analysis of Risk Factors for Necrotizing Enterocolitis in Premature Infants and Clinical Significance of Serum SMOX and ACR

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Abstract

Background: This study aimed to identify risk factors for NEC in preterm infants and evaluate the predictive utility of serum spermine oxidase (SMOX) and acrolein (ACR) levels for the early detection of NEC.

Methods: A nested case–control study was conducted among preterm infants with a gestational age of <34 weeks who were admitted to the Neonatal Department of the Children's Hospital of Soochow University within 24 hours after birth between October 2021 and October 2023. Thirty-two infants who subsequently developed NEC (Bell stage II or higher) were included in the NEC group, while 32 gestational age-matched preterm infants without NEC were randomly selected as controls. For each infant in the NEC group, one gestational age-matched control without NEC was selected using a 1:1 matching strategy, based on admission date, gestational age (±1 week), and birth weight (±200 g). Serum SMOX and ACR concentrations were measured on postnatal days 1,7, 14, and 28. Multivariable logistic regression analysis was performed to identify independent risk factors for NEC. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive performance of SMOX and ACR. A combined prediction model incorporating clinical risk factors and serum biomarkers was subsequently established.

Results: A total of 64 preterm infants were included, with a mean gestational age of 29.1 ± 2.0 weeks and a mean birth weight of 1223.0 ± 298.6 g. Compared with the control group, the NEC group had significantly higher rates of respiratory distress syndrome, sepsis, blood transfusion, and fluid overload during the first three postnatal days (all $P < 0.05$), and the proportion of infants who received early enteral feeding was significantly lower ($P < 0.05$). Serum SMOX concentrations on postnatal day 14 were higher in the NEC group than in the control group (249.2 ± 71.2 vs. 194.0 ± 52.9 U/mL, $P < 0.05$), whereas lower concentrations were observed on day 28 (213.2 ± 60.2 vs. 246.0 ± 63.9 U/mL, $P < 0.05$). Serum ACR concentrations were higher in the NEC group on both postnatal days 14 and 28 (66.1 ± 13.3 vs. 58.8 ± 12.7 pg/mL and 71.1 ± 15.8 vs. 58.7 ± 14.5 pg/mL, respectively; both $P < 0.05$). Multivariable logistic regression identified sepsis, fluid overload during the first three postnatal days, and elevated SMOX and ACR levels on postnatal day 14 as independent risk factors for NEC. A prediction model incorporating these variables yielded an area under the ROC curve (AUC) of 0.870 (95% CI: 0.780–0.960), with a sensitivity of 81.3% and a specificity of 90.3%. The Hosmer–Lemeshow goodness-of-fit test showed no evidence of poor calibration ($P = 0.165$). Internal validation using 1,000 bootstrap resamples yielded a 95% confidence interval of 0.787–0.961. The calibration curve showed agreement between predicted and observed probabilities of NEC.

Conclusion: Sepsis and high fluid overload within three days after admission are independent risk factors for NEC in preterm infants <34 weeks of gestation. SMOX and ACR demonstrate good predictive value for NEC, and combined monitoring of these markers may improve the accuracy of NEC prediction and diagnosis.

Introduction

Necrotizing enterocolitis (NEC) is one of the most severe gastrointestinal emergencies in the neonatal period and is characterised by intestinal inflammation, ischaemia, and varying degrees of intestinal necrosis [1]. Despite substantial advances in perinatal and neonatal intensive care, NEC remains a leading cause of morbidity and mortality among preterm infants. The incidence of NEC ranges from 2% to 5% in the general neonatal population and may reach 11% among very low birth weight infants, with reported mortality rates of 15%–45% [2]. Approximately 40% of affected infants require surgical intervention [3], and survivors are at increased risk of both short- and long-term complications, including short bowel syndrome, intestinal strictures, intestinal adhesions, growth impairment, and neurodevelopmental delay [4,5]. Owing to its nonspecific and often insidious early manifestations, NEC may be difficult to recognize at an early stage and can rapidly progress to intestinal perforation, extensive bowel necrosis, and other life-threatening complications [6]. Therefore, the identification of reliable biomarkers and predictive tools for early NEC detection remains a major clinical priority.

Previous studies have demonstrated that alterations in gut microbial colonisation precede the onset of NEC [7]. Emerging evidence suggests that disturbances in microbiota-associated metabolism, particularly amino acid metabolism, may contribute to NEC pathogenesis [8]. Arginine, a semi-essential amino acid, serves as a key precursor for polyamine biosynthesis: it is converted to ornithine, which is subsequently decarboxylated to putrescine and further converted to spermidine and spermine. Spermine oxidase (SMOX), a key enzyme involved in polyamine catabolism, catalyzes the back-conversion of spermine, and acrolein (ACR), a highly reactive and toxic metabolite generated during this process, have been implicated in oxidative stress, inflammatory responses, and intestinal epithelial barrier dysfunction. Excessive activation of the SMOX–ACR pathway may therefore contribute to intestinal injury and NEC development.

In the present study, we investigated the association of serum SMOX and ACR levels with NEC in preterm infants and evaluated their predictive value in combination with clinical risk factors. To our knowledge, this is among the first studies to prospectively evaluate the application of serum SMOX and ACR as early predictive biomarkers of NEC, with the aim of providing a clinically actionable risk-stratification tool for this vulnerable population.

Materials and methods

Patients

This nested case–control study was conducted at the Department of Neonatology at the Children's Hospital of Soochow University. Preterm infants admitted within 24 hours of birth between October 2021 and October 2023 were screened for eligibility.

The inclusion criteria were: (1) gestational age of less than 34 weeks, and (2) admission within 24 hours of birth.

The exclusion criteria were: (1) congenital intestinal diseases, including intestinal malrotation, congenital intestinal atresia, and Hirschsprung disease; (2) inherited metabolic disorders or chromosomal abnormalities; (3) incomplete clinical data; and (4) hospitalisation for less than 48 hours.

NEC was diagnosed according to the modified Bell's staging criteria, and infants classified as Bell stage II or III were assigned to the NEC group.

For each infant in the NEC group, one control infant without NEC was selected using a 1:1 matching strategy based on admission date, gestational age, and birth weight.

The study protocol was approved by the Medical Ethics Committee of Children's Hospital of Soochow University (Approval No. 2023CS125), which waived the requirement for informed consent.

Laboratory workup

Residual blood specimens obtained after routine clinical laboratory testing were collected on postnatal days 1, 7, and 14 throughout the hospital stay. Sample collection ceased at hospital discharge, death, or eight weeks after birth, whichever occurred first. Serum concentrations of SMOX and ACR were quantified using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturers' instructions.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corporation, Armonk, New York, USA) and R software version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables with a normal distribution were presented as the mean ± standard deviation (SD) and compared using an independent-samples t-test. Non-normally distributed variables are expressed as the median and interquartile range (IQR) and were compared using the Mann–Whitney U test. Categorical variables are presented as frequencies and percentages and were compared using the chi-squared test or Fisher's exact test, as appropriate.

Univariable logistic regression analysis was performed to identify variables associated with NEC. Variables with a *P* value of less than 0.05 in the univariable analysis were entered into a multivariable logistic regression model to identify independent risk factors. A prediction model was subsequently developed based on these independent risk factors and serum biomarkers.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive performance of individual biomarkers and the combined prediction model. The area under the ROC curve (AUC), sensitivity, and specificity were calculated.

Model calibration was assessed using the Hosmer–Lemeshow test and a calibration curve. Internal validation was performed using bootstrap resampling (1,000 repetitions) to evaluate model stability and estimate optimism-corrected performance. A two-sided *P* value of less than 0.05 was considered statistically significant.

Among the 32 infants in the NEC group, two developed NEC prior to postnatal day 14. In these cases, serum samples collected on postnatal day 14 were obtained after NEC onset. Both infants were retained in the primary analysis, as the day-14 sampling time point was selected a priori to characterize the metabolic profile during the early high-risk window for NEC, irrespective of individual onset timing. To assess the potential influence of post-onset measurements on the primary findings, a sensitivity analysis was conducted excluding these two cases.

Results

Baseline clinical data

From October 2021 to October 2023, a total of 368 preterm infants with gestational age < 34 weeks were admitted to the Neonatal Department. After excluding 5 cases with incomplete clinical data, 3 cases with genetic metabolic diseases or chromosomal abnormalities, and 4 cases with confirmed congenital gastrointestinal malformations, 32 preterm infants who met the diagnostic criteria for NEC, Bell stage II or III, were ultimately included. The control group was selected from preterm infants without NEC using a 1:1 matching strategy. In total, 64 preterm infants were included in this study, with a mean gestational age of 29.1 ± 2.0 weeks. There were 37 male infants (57.8%), and the mean birth weight was 1223.0 ± 298.6 g.

Among the NEC group, 25 cases (78.1%) were stage II and 7 cases (21.9%) were stage III. The mean age at NEC onset was25.9 ± 10.7 days. Regarding treatment, 27 cases (84.4%) received conservative medical management, 5 cases (15.6%) underwent surgical intervention, and 1 case (3.1%) died.

There were no significant differences between the NEC and control groups with respect to gestational age, birth weight, sex, the proportion of infants with Apgar scores ≤ 7 at 1 and 5 min, maternal pregnancy complications, or antenatal treatments, including corticosteroids and magnesium sulfate (all $P > 0.05$) (Table 1).

Table 1
Baseline characteristics of the NEC and control groups

Preterm infants	**NEC group (n = 32)**	**Control group (n = 32)**	***P value***
Gestational age [($\bar{x}$±s), weeks]	29.0 ± 1.9	29.2 ± 2.0	0.692
Male [n (%)]	18 (56.3)	19 (59.4)	0.800
Birth weight [($\bar{x}$±s), g]	1213.8 ± 299.5	1232.2 ± 302.1	0.809
1-min Apgar score ≤ 7 [n (%)]	10 (31.3)	10 (31.3)	1.000
5-min Apgar score ≤ 7 [n (%)]	1 (3.1)	3 (9.4)	0.606
Maternal characteristics			
Cesarean section [n (%)]	15 (46.9)	15 (46.9)	1.000
Gestational diabetes [n (%)]	9 (28.1)	10 (31.3)	0.784
Hypertension/preeclampsia [n (%)]	12 (37.5)	8 (25.0)	0.280
PROM > 18 h [n (%)]	10 (31.3)	8 (25.0)	0.578
Chorioamnionitis [n (%)]	1 (3.1)	0 (0.0)	1.000
Antenatal corticosteroid exposure [n (%)]	20 (62.5)	27 (84.4)	0.089
Antenatal magnesium sulfate [n (%)]	4 (12.5)	6 (18.8)	0.491

PROM: prelabor rupture of membranes.

Clinical events occurring before NEC onset were compared between the two groups. The incidences of RDS and sepsis, as well as the proportions of infants receiving blood transfusions, experiencing delayed enteral feeding, and developing fluid overload within the first 3 days after admission(FO3), were significantly higher in the NEC group than in the control group (all $P < 0.05$). However, there were no statistically significant differences in the incidence of SGA, BPD, or the use of mechanical ventilation, PICC, or umbilical venous catheterisation (all $P > 0.05$) (Table 2).

Table 2
Clinical characteristics of the NEC and control groups

SGA [n (%)]	**NEC group (n = 32)**	**Control group (n = 32)**	***P value***
	4 (12.5)	**4 (12.5)**	**1.000**
RDS [n (%)]	27 (84.4)	20 (62.5)	0.048
BPD [n (%)]	15 (46.9)	15 (46.9)	1.000
Sepsis [n (%)]	10 (31.3)	3 (9.4)	0.030
hsPDA [n (%)]	8 (25.0)	3 (9.4)	0.098
Invasive respiratory support [n (%)]	13 (40.6)	14 (43.8)	0.800
Umbilical venous catheterization [n (%)]	11 (34.4)	18 (56.3)	0.079
PICC [n (%)]	30 (93.8)	27 (84.4)	0.423
Blood transfusion [n (%)]	18 (56.3)	10 (31.3)	0.044
Enteral feeding initiation [n (%)]			
Early feeding	2 (6.3)	8 (25.0)	0.039
Delayed feeding (≥ 3 days)	8 (25.0)	6 (18.8)	0.545
Feeding type [n (%)]			1.000
Formula feeding	25 (78.1)	25 (78.1)	
Mixed feeding	7 (21.9)	7 (21.9)	
FO3 [M (IQR), %]	3.7 (1.9–6.2)	1.1 (− 2.2–5.4)	0.006

SGA: small for gestational age; RDS: respiratory distress syndrome; BPD: bronchopulmonary dysplasia; hsPDA: hemodynamically significant patent ductus arteriosus; PICC: peripherally inserted central catheter; FO3: fluid overload within the first 3 days after admission (expressed as %).

Serum SMOX levels on postnatal days 14 and 28 were 249.2 ± 71.2 U/mL and 213.2 ± 60.2 U/mL, respectively, in the NEC group, compared with 194.0 ± 52.9 U/mL and 246.0 ± 63.9 U/mL in the control group. Levels of SMOX were found to be significantly higher in the NEC group than in the control group on postnatal day 14, but significantly lower on postnatal day 28 (both $P < 0.05$).

Serum ACR levels on postnatal days 14 and 28 were 66.1 ± 13.3 pg/mL and 71.1 ± 15.8 pg/mL, respectively, in the NEC group, compared with 58.8 ± 12.7 pg/mL and 58.7 ± 14.5 pg/mL in the control group. ACR levels were found to be significantly higher in the NEC group than in the control group on both postnatal days 14 and 28 (both $P < 0.05$). No significant differences in serum SMOX or ACR levels were observed between the two groups at the remaining time points (all $P > 0.05$) (Table 3).

Table 3
Serum SMOX and ACR levels in the NEC and control groups

SMOX (1d) [($\bar{x}$±s), U/mL]	**NEC group (n = 32)**	**Control group (n = 32)**	***P value***
	240.4 ± 66.3	**245.5 ± 43.8**	**0.716**
SMOX (7d) [($\bar{x}$±s), U/mL]	232.4 ± 61.8	224.7 ± 60.8	0.617
SMOX (14d) [($\bar{x}$±s), U/mL]	249.2 ± 71.2	194.0 ± 52.9	0.001
SMOX (28d) [($\bar{x}$±s), U/mL]	213.2 ± 60.2	246.0 ± 63.9	0.039
ACR (1d) [($\bar{x}$±s), pg/mL]	61.5 ± 13.8	59.5 ± 16.3	0.588
ACR (7d) [($\bar{x}$±s), pg/mL]	61.6 ± 13.5	61.4 ± 15.8	0.958
ACR (14d) [($\bar{x}$±s), pg/mL]	66.1 ± 13.3	58.8 ± 12.7	0.029
ACR (28d) [($\bar{x}$±s), pg/mL]	71.1 ± 15.8	58.7 ± 14.5	0.002

SMOX: spermine oxidase; ACR: acrolein. Days refer to postnatal age.

ROC curve analysis was performed to assess the ability of serum SMOX and ACR levels to predict NEC. Among the biomarkers evaluated, serum SMOX measured on postnatal day 14 demonstrated the optimal predictive performance, with an AUC of 0.745 (95% CI, 0.624–0.866). A cutoff value of 166.8 U/mL yielded a sensitivity of 87.5% and a specificity of 50.0%. The AUCs of serum SMOX on postnatal day 28 and serum ACR on postnatal days 14 and 28 were determined to be 0.647 (95% CI 0.511–0.783), 0.744 (95% CI 0.622–0.866), and 0.709 (95% CI 0.578–0.839), respectively. The corresponding optimal cutoff values were determined to be 236.5 U/mL, 58.2 pg/mL, and 66.2 pg/mL, respectively, with sensitivities of 62.5%, 81.3%, and 68.7%, and specificities of 65.6%, 64.5%, and 25.0%, respectively (Fig. 1).

Figure 1. ROC curves of serum SMOX and ACR levels for predicting NEC

SMOX: spermine oxidase; ACR: acrolein. Days refer to postnatal age.

Variables that were significant in the univariate analysis were included in the multivariable logistic regression model. Sepsis (OR 8.310, 95% CI 1.300–53.105), fluid overload within the first three days after admission (OR 1.184, 95% CI 1.016–1.380), elevated serum SMOX levels on postnatal day 14 (OR 1.016, 95% CI 1.004–1.028), and elevated serum ACR levels on postnatal day 14 (OR 1.076, 95% CI 1.009–1.148) were identified as independent risk factors for NEC (Table 4).

Table 4
Multivariable logistic regression analysis of risk factors for NEC

FO3	B	S.E.	Wald	*P value*	OR	95% CI
	0.169	**0.078**	**4.676**	**0.031**	**1.184**	**1.016–1.380**
Sepsis	2.117	0.946	5.007	0.025	8.310	1.300–53.105
ACR (14d)	0.073	0.033	4.919	0.027	1.076	1.009–1.148
SMOX (14d)	0.016	0.006	6.732	0.009	1.016	1.004–1.028

FO3: fluid overload within the first 3 days after admission; ACR (14d): serum acrolein levels measured on postnatal day 14; SMOX (14d): serum spermine oxidase levels measured on postnatal day 14. The model constant (intercept) is not shown.

Based on the four independent risk factors identified above, a combined prediction model was constructed using multivariable logistic regression. ROC curve analyses were conducted to assess the predictive value of sepsis, FO3, SMOX levels on postnatal day 14, ACR levels on postnatal day 14, as well as this combined prediction model for NEC. The AUC values for sepsis, FO3, SMOX, and ACR were 0.701 (95% CI: 0.567–0.835), 0.608 (95% CI: 0.468–0.748), 0.745 (95% CI: 0.624–0.866), and 0.744 (95% CI: 0.622–0.866), respectively. Notably, although FO3 showed a non-significant univariable AUC (P = 0.141), it was retained as an independent predictor in the multivariable model (P = 0.031), reflecting the fact that univariable ROC analysis and multivariable regression assess different aspects of a variable's contribution: the former evaluates its discriminatory ability in isolation, whereas the latter evaluates its independent effect after adjustment for other covariates. The prediction model demonstrated the highest predictive efficacy, with an AUC of 0.870 (95% CI: 0.780–0.960), a sensitivity of 81.3%, and a specificity of 90.3% (Table 5 and Fig. 2).

The Hosmer–Lemeshow goodness-of-fit test yielded no evidence of poor calibration ($\chi^2 = 5.101$, $P = 0.165$), thereby indicating a satisfactory degree of agreement between predicted and observed probabilities. The internal validation process, which involved the use of 1,000 bootstrap resamples, yielded a 95% CI of 0.787–0.961. Furthermore, the calibration curve showed good agreement between predicted and observed NEC probabilities, indicating satisfactory model calibration and stability (Fig. 3).

Table 5
Predictive performance of independent risk factors and the prediction model for NEC

FO3	AUC	*P value*	95% CI	Sensitivity (%)	Specificity (%)
	0.608	**0.141**	**0.468–0.748**	**31.30**	**90.30**
Sepsis	0.701	0.006	0.567–0.835	90.60	54.80
ACR (14d)	0.744	0.001	0.622–0.866	81.30	64.50
SMOX (14d)	0.745	0.001	0.624–0.866	71.90	67.70
Prediction model	0.870	< 0.001	0.780–0.960	81.30	90.30

FO3: fluid overload within the first 3 days after admission; ACR (14d): serum acrolein levels measured on postnatal day 14; SMOX (14d): serum spermine oxidase levels measured on postnatal day 14.

Figure 2. ROC curves of independent risk factors and the prediction model for predicting NEC

FO3: fluid overload within the first 3 days after admission; ACR (14d): serum acrolein levels measured on postnatal day 14; SMOX (14d): serum spermine oxidase levels measured on postnatal day 14.

Discussion

In the present study, sepsis and fluid overload within the first three postnatal days were identified as independent clinical risk factors for NEC, and serum SMOX and ACR measured on postnatal day 14 were shown to provide additional predictive value beyond clinical variables alone. The development of NEC is influenced by multiple factors, including the intrauterine environment, perinatal conditions, and postnatal diseases [9–10]. Although the pathogenesis of NEC remains incompletely understood, increasing evidence suggests that intestinal immaturity, dysbiosis, impaired microcirculation, exaggerated inflammatory responses, and genetic susceptibility contribute to disease development [11]. In recent years, the role of amino acid metabolism in NEC has attracted considerable attention; however, the involvement of polyamine metabolism and its downstream metabolites remains to be fully elucidated. SMOX and ACR may aggravate intestinal bacterial translocation, intestinal inflammatory injury, and epithelial barrier dysfunction through oxidative stress, inflammatory responses, and immune dysregulation, all of which are key processes in the initiation and progression of NEC [12–14].

In the present study, the proportion of infants receiving blood transfusions was significantly higher in the NEC group than in the control group, which is consistent with previous reports [15]. It has been hypothesised that the presence of anaemia itself may result in impaired intestinal perfusion, while subsequent red blood cell transfusion may trigger intestinal mucosal immune responses and ischemia–reperfusion injury, thereby increasing the risk of NEC [15]. Furthermore, a series of pathological changes may occur during blood product storage, including reduced erythrocyte deformability, increased oxygen affinity, and decreased nitric oxide bioavailability. These alterations have the potential to compromise

intestinal microcirculatory blood flow following transfusion, thereby contributing to the development of NEC [16]. However, multivariable analysis revealed that blood transfusion was not identified as an independent risk factor. This discrepancy may be attributable to the relatively modest sample size of the present study, which may have constrained the statistical power to discern an independent association between transfusion exposure and NEC.

Sepsis is an acute systemic infection caused by the invasion of pathogenic microorganisms into the bloodstream. It is characterized by a dysregulated host immune response and may directly affect the intestine, resulting in intestinal injury. Pathogens and their endotoxins can activate intestinal Toll-like receptor 4 signaling, leading to increased expression of proinflammatory cytokines and suppression of reparative pathways, thereby causing intestinal mucosal damage [10]. In addition, sepsis is frequently accompanied by intestinal dysbiosis, which disrupts host–microbiota homeostasis and contributes to epithelial injury, impaired mucus barrier function, and immune dysregulation, further exacerbating intestinal damage [17]. In the present study, sepsis was identified as an independent risk factor for NEC, consistent with the findings of Cotton et al [18]. These findings highlight the importance of early recognition, prompt intervention, and comprehensive management of sepsis in reducing the risk of NEC among preterm infants.

Dynamic monitoring of fluid balance during the first three days after admission demonstrated that the cumulative fluid load was consistently higher in the NEC group than in the control group, with the greatest difference observed during this early post-admission period. Fluid therapy has long been recognized as a cornerstone of supportive care in critically ill neonates. Timely and appropriate fluid resuscitation can rapidly restore effective circulating volume, maintain hemodynamic stability, and improve tissue perfusion. However, beyond the fluids required for resuscitation, critically ill infants frequently receive additional volumes for medication administration, nutritional support, and maintenance therapy, resulting in fluid intake that may exceed their capacity for fluid elimination [19]. Growing evidence suggests that fluid overload is not merely a marker of disease severity but may also contribute directly to adverse clinical outcomes. Excessive fluid accumulation can promote interstitial edema, exacerbate organ congestion, and impair microcirculatory perfusion. These pathophysiological alterations may increase susceptibility to NEC through several interrelated mechanisms. Intestinal wall edema may elevate intramural pressure and compromise the mucosal barrier, while tissue congestion and microcirculatory dysfunction can reduce intestinal perfusion and oxygen delivery. In addition, pulmonary edema secondary to fluid overload may aggravate systemic hypoxemia, further exacerbating intestinal ischemia and injury [20]. Collectively, these mechanisms may create a permissive environment for the development and progression of NEC. Taken together, our findings suggest that fluid overload may represent a potentially modifiable risk factor for NEC in preterm infants. Careful monitoring of fluid balance and implementation of individualized fluid management strategies during the early postnatal period may help maintain fluid homeostasis and reduce the risk of NEC and other fluid overload-related complications.

As a key rate-limiting enzyme in the polyamine metabolic pathway, SMOX is involved in a wide range of biological processes, including apoptosis, cell proliferation, and DNA damage repair, and has been implicated in cell cycle regulation, tumorigenesis, inflammatory responses, and neurodegenerative disorders [21]. SMOX is markedly upregulated in response to pathological stimuli such as infection, neuronal excitotoxicity, ischemia, and oxidative stress. It catalyzes the oxidation of spermine to spermidine, concomitantly generating hydrogen peroxide and 3-aminopropanal (3-AP) [13]. Subsequently, 3-AP undergoes spontaneous intracellular conversion to acrolein (ACR), a highly reactive aldehyde that binds DNA and inactivates cellular proteins, thereby contributing to cellular injury and tissue damage. Previous studies have shown that SMOX expression is significantly increased in murine models of colitis and is positively associated with the severity of intestinal injury, whereas pharmacological inhibition of SMOX attenuates intestinal inflammation and tissue damage [22]. Furthermore, under pathological conditions such as ischemia and ischemia–reperfusion injury, aberrant activation of SMOX promotes the release of polyamine metabolites into the circulation, triggering a metabolic cascade that further amplifies systemic SMOX activity and ACR production [23].

In the present study, serial measurements of serum SMOX and ACR at multiple postnatal time points revealed significantly higher SMOX concentrations in infants who subsequently developed NEC compared with controls at postnatal day 14. In contrast, significant differences in ACR levels were observed at both postnatal days 14 and 28. These temporal alterations may reflect early metabolic dysregulation, oxidative stress, and intestinal ischemia–hypoxia that precede the clinical onset of NEC. The relatively delayed elevation of ACR may be biologically plausible given its downstream position in the SMOX-mediated polyamine oxidation pathway. Logistic regression analysis further identified elevated SMOX and ACR levels at postnatal day 14 as independent predictors of NEC. Receiver operating characteristic analysis demonstrated moderate predictive performance, with AUC values of 0.745 for SMOX and 0.744 for ACR. SMOX exhibited a sensitivity of 87.5% and a specificity of 50.0%, whereas ACR showed a sensitivity of 81.3% and a specificity of 64.5%. Collectively, these findings suggest that dynamic monitoring of SMOX and ACR may provide a useful approach for the early identification of preterm infants at increased risk of NEC, although the precise molecular mechanisms linking polyamine metabolism to NEC pathogenesis require further elucidation.

Several limitations of this study should be acknowledged. First, this was a single-center study with a relatively small sample size, which may have limited the statistical power of the analyses. Second, although the prediction model was internally validated using bootstrap resampling and demonstrated satisfactory calibration, it has not undergone external validation in an independent cohort, which precludes a comprehensive assessment of its generalisability and clinical utility. Therefore, larger prospective multicenter studies with external validation are needed to confirm these findings and further establish the clinical value of SMOX and ACR as biomarkers for NEC prediction.

Conclusion

In conclusion, serum SMOX and ACR demonstrated moderate but clinically meaningful predictive performance for NEC in preterm infants. Dynamic monitoring of these biomarkers may facilitate early risk stratification, and their integration with established clinical risk factors may improve the identification of infants at increased risk for NEC. Further multicenter prospective studies with external validation are required to confirm these findings and clarify the underlying biological mechanisms.

Declarations

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Data availability

Availability of data and materials. The novel findings and contributions of this research are documented in the manuscript and the appendix data. For any additional questions, please contact the corresponding author.

Author contributions

SX: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.CW: Investigation, Data curation, Writing – review & editing.XZ: Supervision, Funding acquisition, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Clinical trial number: not applicable

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Figures

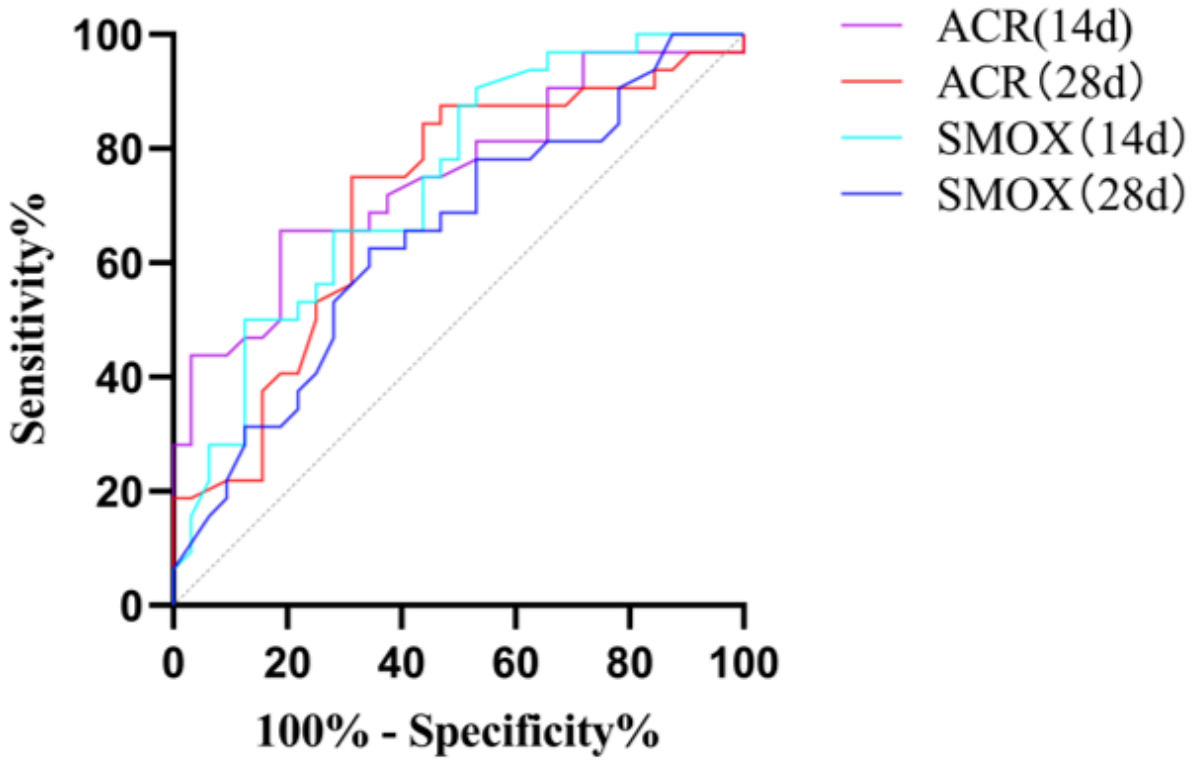


Figure 1

ROC curves of serum SMOX and ACR levels for predicting NEC

SMOX: spermine oxidase; ACR: acrolein. Days refer to postnatal age.

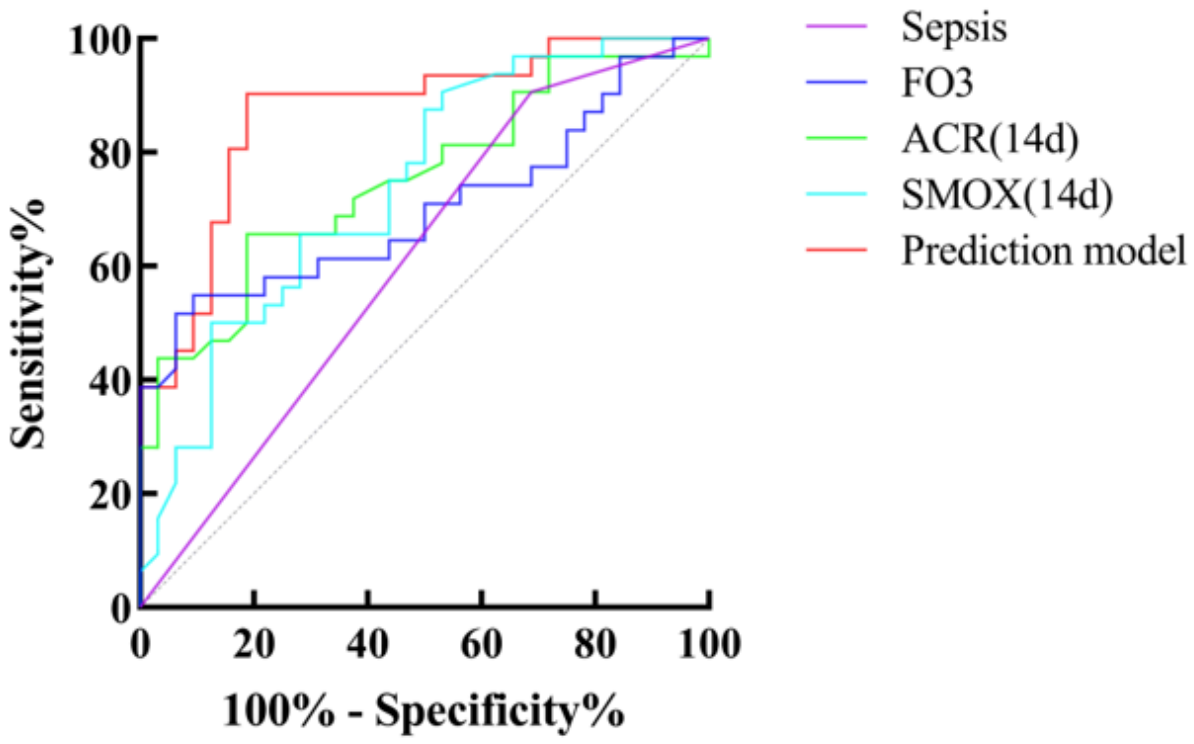


Figure 2

ROC curves of independent risk factors and the prediction model for predicting NEC

FO3: fluid overload within the first 3 days after admission; ACR (14d): serum acrolein levels measured on postnatal day 14; SMOX (14d): serum spermine oxidase levels measured on postnatal day 14.

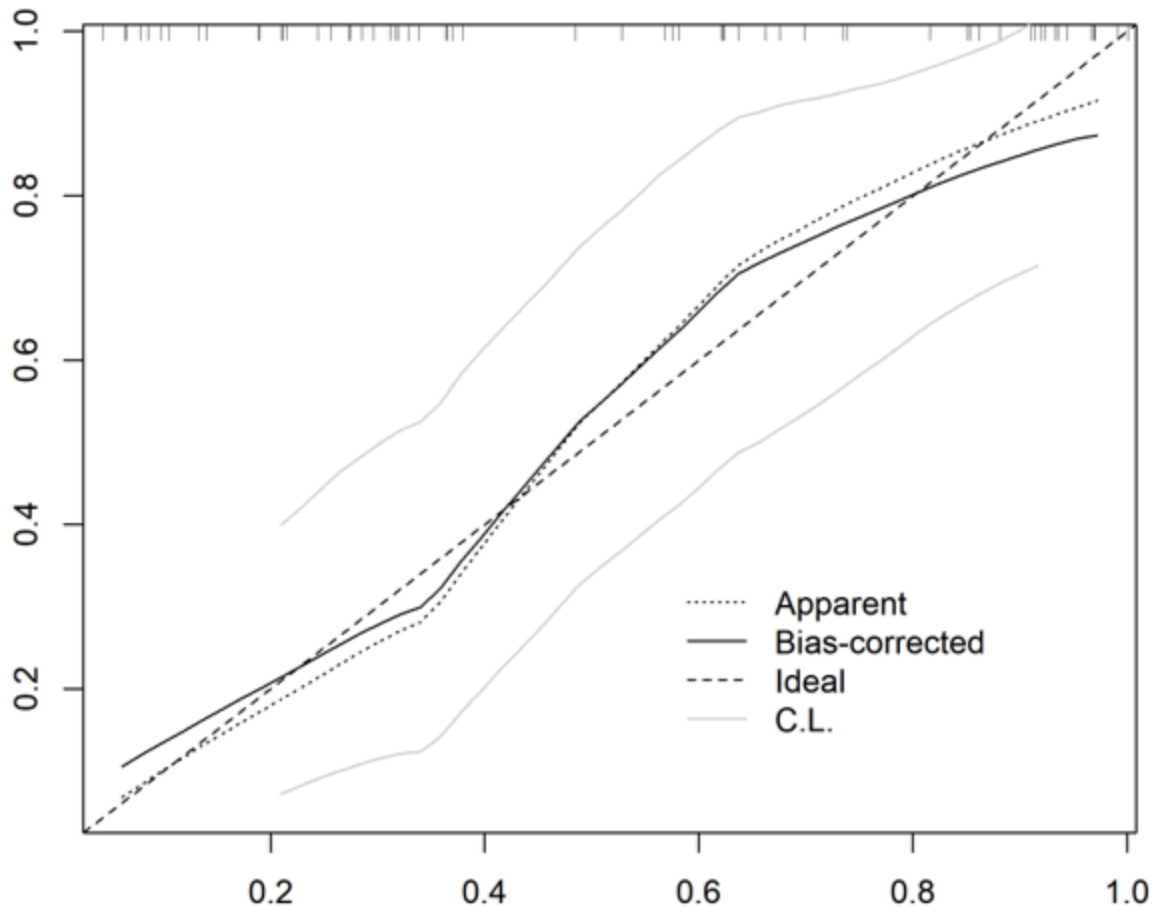


Figure 3

Calibration curve of the NEC prediction model