

Incidence of necrotizing enterocolitis and predisposing factors in extremely preterm infants: A single-center experience

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ABSTRACT

Objective: Necrotizing enterocolitis (NEC) remains a major cause of morbidity and mortality in extremely preterm infants. This study aimed to determine the incidence of NEC, identify antenatal, perinatal, and postnatal risk factors, and evaluate predictors of disease severity and mortality in infants born at ≤ 32 weeks' gestation.

Materials and Methods: In this retrospective single-center study, 1509 preterm infants admitted to the NICU of Ankara Bilkent City Hospital between 2019 and 2024 were reviewed. Infants with stage II–III NEC (n=109) were compared with gestational age matched controls (n=252). Clinical variables, including feeding characteristics, laboratory markers, complications, and outcomes were analyzed using logistic regression and ROC analysis.

Results: NEC was strongly associated with lower gestational age, birth weight < 1000 g, low APGAR scores, need for resuscitation, umbilical catheterization, PDA, transfusion, and delayed initiation of enteral feeding (all $p < 0.001$). Human milk fortifier use was associated with reduced NEC risk. Late onset sepsis, metabolic acidosis, anemia, intracranial hemorrhage, and inotrope use independently predicted advanced stage NEC. Pre diagnostic IL 6 levels were higher in stage III NEC. Gestational age ≤ 29.5 weeks and birth weight ≤ 1235 g were moderate predictors of severe NEC. Stage III NEC was associated with significantly higher complication and mortality rates.

Conclusion: Extreme prematurity, hemodynamic instability, delayed feeding, and inflammatory activation are major determinants of NEC severity and mortality. Early risk stratification and optimized feeding strategies may reduce NEC related morbidity and mortality.

Keywords: Mortality, necrotizing enterocolitis, neonatal intensive care, premature, risk factors

Introduction

Necrotizing enterocolitis (NEC) is one of the most serious gastrointestinal emergencies of the neonatal period, primarily affecting preterm infants and associated with substantial morbidity and mortality. Despite decades of research, the exact etiopathogenesis of NEC remains incompletely understood. Current evidence suggests that multiple interacting mechanisms contribute to disease development, including intestinal immaturity related to prematurity, microbial dysbiosis, ischemiareperfusion injury, disruption of epithelial barrier integrity, and an exaggerated inflammatory response (1-3).

The clinical presentation of NEC varies widely, ranging from mild feeding intolerance and abdominal distension to fulminant disease with rapid systemic deterioration (4-6). Therefore, diagnosis often requires integration of clinical findings with radiological and laboratory parameters. The modified Bell staging system is widely used to classify disease severity and guide management (7). However, most

diagnostic tools are applied after disease onset, and early identification of infants at high risk for NEC remains challenging.

Despite advances in neonatal intensive care and surgical techniques, outcomes of NEC remain suboptimal. Survivors frequently experience longterm complications such as intestinal failure, growth restriction, and neurodevelopmental impairment, emphasizing the need for preventive strategies and early risk stratification (3). Among the many proposed risk factors, prematurity is the most consistently identified and potentially modifiable determinant (8). Infants born at < 32 weeks' gestation are particularly vulnerable due to immature intestinal barrier function, impaired immune regulation, and abnormal microbial colonization.

In this context, the present study aimed to determine the incidence of NEC in infants born at ≤ 32 weeks' gestation in our center, to evaluate antenatal, perinatal, and postnatal risk factors associated with NEC development, to assess feeding practices and laboratory predictors, and to identify clinical factors associated with disease severity and mortality.

Materials and Methods

Study design and population

This retrospective singlecenter study was conducted in the Neonatal Intensive Care Unit (NICU) of Ankara Bilkent City Hospital between 1 January 2019 and 31 December 2024. During this period, 1509 infants with gestational age ≤ 32 weeks were admitted and screened for eligibility.

Infants were divided into two groups: NEC group: Infants diagnosed with stage II or stage III NEC according to the Modified Bell criteria ($n=109$); Control group: Preterm infants without NEC matched for gestational age period ($n=252$). Control infants were selected from the same NICU population during the same study period and were matched for gestational age (± 1 week) and birth weight (± 100 g) to minimize confounding related to prematurity. Infants with spontaneous intestinal perforation, congenital gastrointestinal anomalies, incomplete records, or stage I (suspected) NEC were excluded.

Data collection

Clinical data were extracted from electronic hospital records. The following variables were recorded: Antenatal Variables: Maternal age, gravidity, maternal smoking, chorioamnionitis, premature rupture of membranes (PROM), placental abruption, maternal anemia, preeclampsia/eclampsia, gestational diabetes mellitus, antenatal corticosteroid administration, mode of delivery.

Perinatal Variables: Gestational age, birth weight, 1 and 5 minute APGAR scores, need for resuscitation, surfactant use, intubation/mechanical ventilation, umbilical arterial catheter (UAC) and venous catheter (UVC) use were recorded.

Postnatal Variables: including respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), retinopathy of prematurity (ROP), intracranial hemorrhage, neonatal anemia, lateonset neonatal sepsis, blood transfusion, inotrope use, antibiotic therapy duration were recorded.

Feeding characteristics: Day of initiation of enteral feeding, rate of feed advancement, time to full enteral feeding, use of human milk fortifier, feeding intolerance were recorded.

Definitions of key variables were as follows:

- Metabolic acidosis was defined as a base deficit ≥ 10 mmol/L or pH < 7.20 on blood gas analysis requiring bicarbonate therapy.
- Neonatal anemia was defined as hemoglobin level below the 10th percentile for gestational and postnatal age, requiring packed red blood cell transfusion.
- Delayed initiation of enteral feeding was defined as initiation of enteral feeding after 72 hours of life.
- Lateonset sepsis was defined as positive blood or cerebrospinal fluid culture obtained after 72 hours of life with associated clinical signs of infection.
- Inotrope use was defined as administration of dopamine, dobutamine, or epinephrine for hemodynamic support.
- Feeding intolerance was defined as the presence of gastric residuals $> 50\%$ of the previous feed volume, abdominal distension, or emesis requiring modification of the feeding plan.

NEC Specific Data: For infants diagnosed with NEC, additional variables were recorded: Day of NEC onset, Bell stage (II or III), Laboratory parameters (WBC, platelet count, CRP, IL6) before onset and at diagnosis, Radiological findings (abdominal radiography, ultrasonography), Antibiotic treatment, Surgical interventions (peritoneal drainage, laparotomy), Duration of interruption of enteral feeding were recorded.

Outcome Measures

The primary outcomes of the study were the incidence of necrotizing enterocolitis (NEC) and the identification of clinical risk factors associated with stage II and stage III NEC according to the Modified Bell criteria. Secondary outcomes included all cause mortality and NEC related complications such as late onset sepsis, enterostomy requirement, intestinal stricture, recurrent NEC, intracranial hemorrhage, postoperative adhesions, intestinal or hepatic failure, short bowel syndrome, growth failure, and neurodevelopmental impairment. In addition, laboratory predictors of disease severity, including inflammatory markers measured before and at the time of NEC diagnosis, and clinical parameters such as feeding characteristics, transfusion requirement, and inotrope use were evaluated to determine their association with advanced stage NEC and mortality.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean and standard deviation or median (interquartile range) according to distribution, and categorical variables as number and percentage. Normality of distribution was assessed using Kolmogorov Smirnov and ShapiroWilk tests. Comparisons between groups were performed using the chisquare test or Fisher's exact test for categorical variables and Student's t test, Mann Whitney U test, or Kruskal Wallis test for continuous variables as appropriate. Variables with $p < 0.100$ in univariable analyses were entered into multivariable binary logistic regression models to identify independent predictors of stage II NEC, stage III NEC, and mortality. To address potential collinearity, variance inflation factor (VIF) analysis was performed, and variables with VIF > 5 were excluded from the final models. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. Receiver operating characteristic (ROC) curve analysis was performed to determine optimal cut off values for gestational age, birth weight, IL6, and CRP in predicting severe NEC. Area under the curve (AUC) values with 95% CI, sensitivity, and specificity were calculated for each ROC analysis. A two sided p value < 0.050 was considered statistically significant.

Results

During the study period, 1509 preterm infants with gestational age ≤ 32 weeks were admitted to the NICU. Among them, 222 infants were diagnosed with NEC (14.7%), including stage I (7.6%), stage II (3.5%), and stage III (3.6%). After exclusion criteria were applied, 361 infants were included in the final analysis: 54 infants with stage II NEC, 55 with stage III NEC, and 252 controls. Baseline antenatal, perinatal, and postnatal characteristics are summarized in Table I.

Table I: Antenatal, natal, and postnatal characteristics of the mothers and infants, and results of between-group comparisons

	Control Group	NEC		p
		Stage 2	Stage 3	
Total number of patients	252	54	55	-
Maternal characteristics				
Maternal age*	29.40±5.05 (17-47)	27.80±5.82 (18-40)	29.98±6.53 (17-44)	0.098
Gravidity*	2.31±1.60 (1-12)	2.20±1.26 (1-6)	2.49±1.45 1-7)	0.413
History of maternal smoking†	10 (4.0)	1 (1.9)	3 (5.5)	0.617
Presence of chorioamnionitis†	24 (9.5)	1 (1.9)	5 (9.1)	0.175
Presence of placental abruption†	8 (3.2)	3 (5.6)	2 (3.6)	0.695
Presence of PROM†	47 (18.7)	1 (1.9)	12 (21.8)	0.006
Presence of maternal anaemia†	71 (28.2)	21 (38.9)	18 (32.7)	0.277
Presence of eclampsia†	36 (14.3)	11 (20.4)	5 (9.1)	0.244
Presence of GDM†	27 (10.7)	4 (7.4)	3 (5.5)	0.414
Administration of antenatal corticosteroids†	173 (68.7)	34 (63.0)	34 (61.8)	0.506
Mode of delivery†				
Vaginal delivery	32 (12.7)	7 (13.0)	9 (16.4)	0.766
Caesarean section (C/S)	220 (87.3)	47 (87.0)	9 (83.6)	
Presence of hypoxia/asphyxia†	14 (5.6)	6 (11.1)	11 (20.0)	0.002
Gestational age†				
<23 weeks	11 (4.4)	1 (1.9)	3 (5.5)	<0.001
23–28 weeks	64 (25.4)	27 (50.0)	29 (52.7)	
28–32 weeks	176 (70.2)	26 (48.1)	23 (41.8)	
Birth weight†				
<1.000 g	62 (24.6)	29 (53.7)	29 (52.7)	<0.001
1.000–1.500 g	88 (35.3)	20 (37.0)	19 (34.5)	
1.500–2.500 g	101 (40.1)	5 (9.3)	7 (12.7)	
APGAR score at 1 minute‡	6 (4-6)	4 (3-5)	4 (2-5)	<0.001
APGAR score at 5 minutes‡	8 (6-8)	6 (5-7)	6 (5-7)	<0.001
Need for resuscitation at birth†	116 (46.0)	36 (66.7)	39 (72.2)	<0.001
Need for surfactant†	123 (48.8)	40 (74.1)	42 (76.4)	<0.001
Need for intubation†	126 (50.0)	44 (81.5)	49 (89.1)	<0.001
Umbilical arterial catheterisation†	38 (15.1)	15 (27.8)	29 (52.7)	<0.001
Presence of umbilical venous catheter†	155 (61.5)	46 (85.2)	45 (81.8)	<0.001
Presence of RDS†	211 (83.7)	54 (100.0)	51 (92.7)	0.002
Presence of BPD†	26 (10.3)	8 (14.8)	9 (16.4)	0.358
Presence of PDA†	103 (40.9)	33 (61.1)	37 (67.3)	<0.001
Presence of ROP†	64 (25.4)	16 (29.6)	16 (29.1)	0.735
Presence of intracranial haemorrhage†	85 (33.7)	27 (50.0)	31 (56.4)	0.002
Presence of neonatal anaemia†	90 (35.7)	48 (88.9)	47 (85.5)	<0.001
Blood culture positivity†	10 (4.0)	46 (85.2)	37 (67.3)	<0.001
Plain abdominal radiography finding(s)†	3 (1.2)	52 (96.3)	55 (100)	<0.001
Ibuprofen use†	21 (8.3)	8 (14.8)	10 (18.2)	0.061
Paracetamol (acetaminophen) use†	33 (13.1)	21 (38.9)	22 (40.0)	<0.001
Antibiotic use	209 (82.9)	54 (100)	55 (100)	<0.001
Inotrope use†	47 (18.7)	28 (51.9)	42 (76.4)	<0.001
Caffeine use†	182 (72.2)	47 (87.0)	50 (90.9)	0.002
Total duration of antibiotic use (days)‡	7 (2-15)	23 (16.7-35)	24 (12-38)	<0.001
Need for transfusion†	68 (27.0)	41 (75.9)	48 (87.3)	<0.001
Time to initiation of enteral feeds‡	1 (1-2)	2 (1-3)	2 (1-3)	<0.001
Rate of feed advancement (mL/kg/day)‡	24 (12-24)	12 (12-12)	12 (12-12)	<0.001
Day of achieving full enteral feeding‡	11 (8-15.2)	26 (20.5-39)	28 (20-42)	<0.001
TPN (total parenteral nutrition)	232 (92.5)	53 (98.1)	55 (100)	0.160
Duration of TPN (days)‡	9 (5-14)	25.5 (20-37.2)	23 (12-42)	<0.001
Presence of feeding intolerance†	57 (22.6)	23 (42.6)	17 (30.9)	0.042

*: mean±SD (min–max) (Student T Test), †: n (%) (Chi-square test) ‡: median (Q1–Q3) (Kruskal–Wallis test, post-hoc Bonferroni test)

Infants who developed NEC had significantly lower gestational age and birth weight compared with controls ($p<0.001$). NEC incidence was particularly high in infants born at 23–28 weeks' gestation and with birth weight <1000 g. Both stage II and stage III NEC groups had significantly lower 1-minute

and 5 minute APGAR scores, higher need for resuscitation, surfactant therapy, and intubation compared with controls (all $p<0.001$). PROM was significantly more frequent in stage III NEC ($p=0.006$), while maternal characteristics such as

Table II: Evaluation of risk factors for stage II NEC using univariable and multivariable analyses

Variable	Univariable analyses		Multivariable analyses	
	OR (95% CI)	p	OR (95% CI)	p
Presence of premature rupture of membranes (PROM)	0.082 (0.011-0.610)	0.015	0.059 (0.005-0.652)	0.021
Presence of metabolic acidosis	13.375 (6.787-26.358)	<0.001	14.404 (3.761-55.169)	<0.001
Presence of late-onset neonatal sepsis	25.915 (9.051-74.201)	<0.001	67.571 (10.737-425.262)	<0.001
Presence of intracranial haemorrhage	1.965 (1.085-3.558)	0.026	3.835 (1.326-11.087)	0.013
Presence of anaemia	14.400 (5.932-34.959)	<0.001	5.662 (1.616-19.833)	0.007

OR: odds ratio, CI: confidence interval

Table III: Evaluation of risk factors for stage III NEC using univariable and multivariable analyses

Variable	Univariable analyses		Multivariable analyses	
	OR (95% CI)	p	OR (95% CI)	p
Presence of metabolic acidosis	45.991 (18.416-114.858)	<0.001	23.272 (4.597-117.823)	<0.001
Presence of late-onset neonatal sepsis	16.931 (6.968-41.136)	<0.001	9.432 (2.256-39.435)	0.002
Inotrop use	14.092 (7.011-28.325)	<0.001	6.805 (1.311-35.333)	0.022

OR: odds ratio, CI: confidence interval

Table IV: Distribution of complications observed in infants with NEC.

NEC complications	Stage II*	Stage III*	p
Sepsis	49 (90.7)	41 (74.5)	0.026
Enterostomy	3 (5.6)	12 (21.8)	0.014
Stricture	8 (14.8)	6 (10.9)	0.542
Recurrent NEC	14 (25.9)	19 (34.5)	0.327
Intracranial haemorrhage	25 (46.3)	24 (43.6)	0.780
Adhesions	5 (9.3)	5 (9.1)	0.976
Intestinal/hepatic failure	4 (7.4)	19 (34.5)	<0.001
Growth failure	9 (16.7)	20 (36.4)	0.020
Neurodevelopmental impairment	7 (13.2)	18 (32.7)	0.016
Short bowel syndrome	4 (7.4)	23 (41.8)	<0.001
Peritoneal drainage	5 (9.3)	33 (60.0)	<0.001
Laparotomy	14 (25.9)	34 (61.8)	<0.001

*: n(%) (chi-square test)

maternal age, gravidity, smoking, and gestational diabetes were not significantly different between groups (Table I).

Umbilical arterial and venous catheterization, RDS, PDA, intracranial hemorrhage, neonatal anemia, prolonged antibiotic use, inotrope requirement, and blood transfusion were significantly more frequent in NEC groups compared with controls ($p<0.001$) (Table I). Lateonset neonatal sepsis was observed in 92.6% of stage II NEC and 89.1% of stage III NEC patients, compared with 32.5% in controls ($p<0.001$). The slightly higher sepsis rate observed in stage II compared with stage III may reflect the influence of additional risk factors and competing morbidities in the advanced stage group, where higher mortality prior to sepsis diagnosis could contribute to this difference. Feeding practices differed significantly between groups. NEC infants had delayed initiation of enteral feeding, slower feed advancement, and later achievement of full enteral feeding ($p<0.001$) (Table I).

Logistic regression analysis was performed. For Stage II NEC, in univariable analysis, metabolic acidosis, late onset sepsis, intracranial hemorrhage, and neonatal anemia were associated with increased risk of stage II NEC, whereas PROM was protective. In multivariable analysis, metabolic acidosis (OR=14.4), lateonset sepsis (OR=67.6), intracranial hemorrhage

(OR=3.8), and anemia (OR=5.7) remained independent predictors (Table II). For Stage III NEC, metabolic acidosis, lateonset sepsis, and inotrope use were significant predictors of stage III NEC. In multivariable analysis, metabolic acidosis was associated with a 23 fold increase in risk, lateonset sepsis with a 9.4 fold increase, and inotrope use with a 6.8 fold increase (Table III). The high odds ratios observed in the multivariable models, particularly for late onset sepsis in stage II NEC, warrant caution in interpretation, as these may indicate model instability due to the retrospective nature of the study, small subgroup sizes, or potential collinearity among variables.

The complications observed in NEC patients are summarized in Table IV. Stage III NEC was associated with significantly higher rates of: Enterostomy, intestinal/hepatic failure, growth failure, neurodevelopmental impairment, short bowel syndrome, and surgical intervention. Sepsis was the most frequent complication in both NEC groups.

Laboratory findings including prediagnostic IL6 levels were significantly higher in stage III NEC compared with controls ($p=0.007$). CRP and WBC values differed between groups prior to NEC onset, suggesting early inflammatory activation. At the time of NEC diagnosis, IL6 and CRP levels were significantly higher and platelet counts were lower in stage III NEC compared with stage II NEC (Table IV).

In stage II NEC, univariable analysis showed that gestational age 23-28 weeks, UAC use, metabolic acidosis, inotrope requirement, transfusion, and absence of human milk fortifier were associated with mortality. In multivariable analysis, inotrope use remained the strongest predictor of death. In stage III NEC, mortality was higher among infants with birth weight <1000 g. A higher mortality rate was observed in patients requiring inotropes (OR 12.6). A positive abdominal ultrasonography finding was associated with decreased mortality in the multivariable analysis.

ROC analysis demonstrated that gestational age ≤ 29.5 weeks (AUC: 0.642, 95% CI: 0.568-0.716, sensitivity: 61.1%, specificity: 62.7%) and birth weight ≤ 1235 g (AUC: 0.695, 95% CI: 0.623-0.767, sensitivity: 64.8%, specificity: 61.9%) were moderate predictors of stage The optimal cutoff values for predicting stage III NEC were 29.5 weeks for gestational age (AUC= 0.703, 95% CI: 0.636-0.771; sensitivity=74.5%; specificity=72.7%; $p<0.001$) and 1235 g for birth weight (AUC=0.707, 95% CI:

Table V: Comparison of laboratory values across groups.

	Control group*	Stage 2 NEC*	Stage 3 NEC*	p
Prior to the NEC				
IL-6 (pg/mL)	16 (7-47.7)	27 (11-55.5)	35 (11-60)	0.007
CRP (mg/L)	1 (0-2)	0 (0-1)	0 (0-20)	<0.001
WBC ($\times 10^9/L$)	8.48 (6.27-11.76)	6.7 (4.87-9.13)	7.58 (5.5-11.6)	0.010
PLT ($\times 10^9/L$)	240.5 (181-307.8)	211.1 (148.5-282.5)	214 (168-297)	0.083
At the time of NEC				
IL-6 (pg/mL)		61.5 (12-456.2)	226 (38-1000)	0.040
CRP (mg/L)		3 (1-11.2)	10 (2-62)	0.030
WBC ($\times 10^9/L$)		9.88 (6.45-14.59)	8.75 (5.35-15.64)	0.778
PLT ($\times 10^9/L$)		142 (67.25-320.5)	107 (43-175)	0.040

*: median (Q1–Q3) (Kruskal–Wallis test), **WBC**: white blood cell, **CRP**: C-reactive protein, **IL-6**: Interleukin-6. post-hoc Bonferroni test, $p < 0.050$

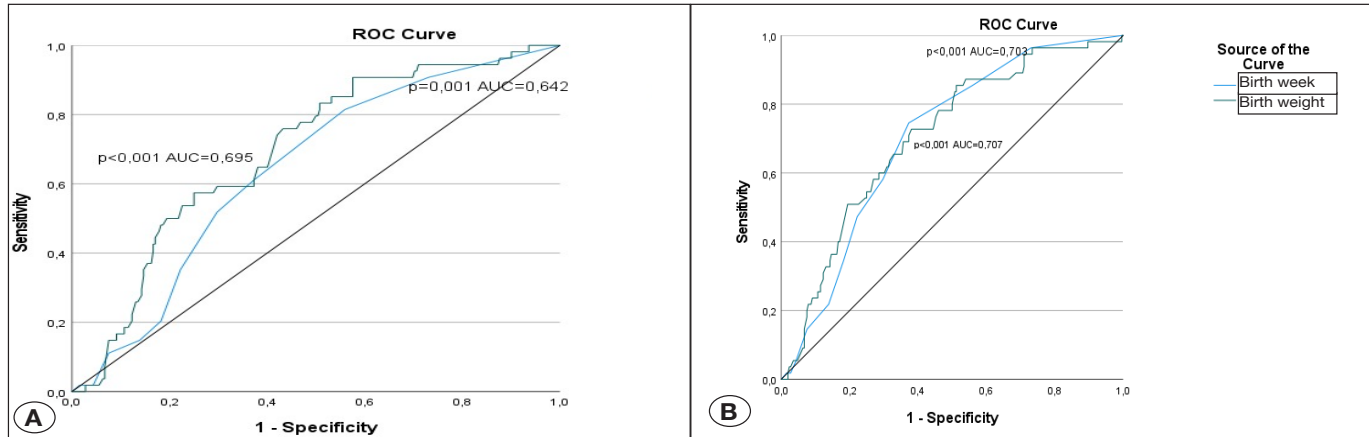


Figure 1: ROC analysis results demonstrating the role of gestational age and birth weight in predicting stage II (A) and stage III (B) disease.

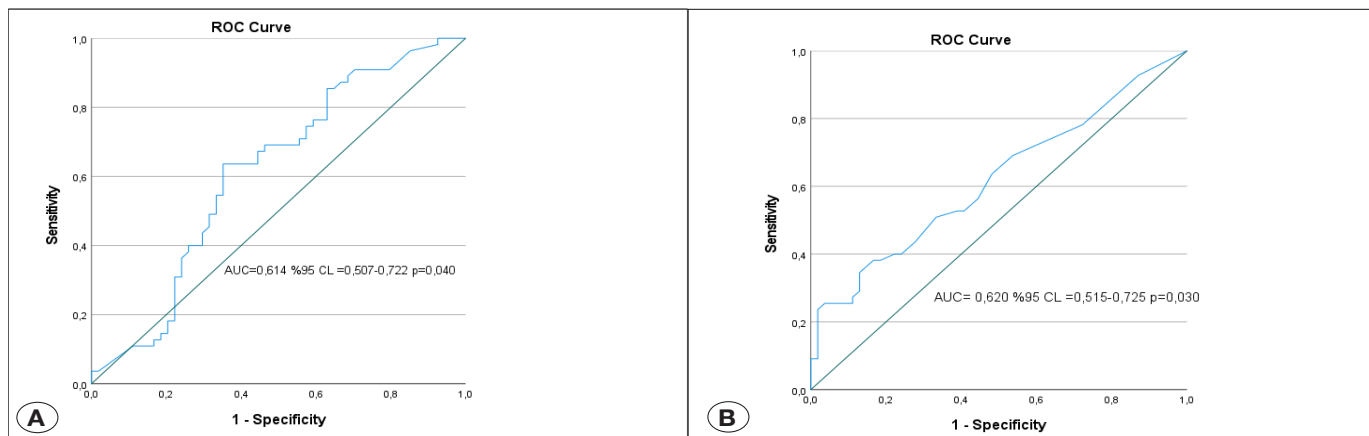


Figure 2: ROC analysis results for IL-6 (A) and CRP (B) values used to predict stage II and stage III disease.

0.635–0.779; sensitivity=69.1%; specificity=64.7%; $p < 0.001$). (Figure 1). An IL6 cut off of 101.5 pg/mL (AUC: 0.614, 95% CI: 0.507–0.722, sensitivity:65.5%, specificity:65.6%) and CRP cutoff of 5 mg/L (AUC: 0.62, 95% CI: 0.515–0.725, sensitivity: 56.4%, specificity: 55.6%) modestly differentiated stage II from stage III NEC (Figure 2).

Discussion

In this retrospective cohort of very preterm infants, NEC was associated with lower gestational age and birth weight, greater neonatal morbidity, and increased need for intensive care support. Metabolic acidosis and late-onset sepsis were independently associated with both stage II and stage III

NEC, while inotrope requirement was particularly associated with advanced disease and mortality. Stage III NEC was also characterized by more severe complications and greater inflammatory activation, with higher IL-6 and CRP levels and lower platelet counts. Overall, these findings highlight the importance of systemic inflammation and hemodynamic instability in NEC severity and prognosis.

Necrotizing enterocolitis remains one of the most devastating complications of prematurity and continues to be associated with significant morbidity and mortality despite advances in neonatal care. Current clinical guidelines and consensus statements emphasise that NEC pathogenesis is multifactorial, involving intestinal immaturity, dysbiosis,

hypoxia ischaemia, and inflammatory activation (17). The lack of a universally accepted definition of NEC also complicates clinical research and risk stratification, and alternative concepts such as hypoxic ischaemic enterocolitis have been proposed to better describe early disease phenotypes (2,3). In this context, identifying modifiable and nonmodifiable risk factors remains crucial for prevention and early diagnosis.

Prematurity is consistently recognised as the strongest determinant of NEC development. Earlier epidemiological and clinical studies demonstrated that decreasing gestational age is associated with earlier disease onset, more subtle clinical presentation, and higher mortality (6,8). In our cohort, infants born at 23-28 weeks constituted approximately half of both stage II and stage III NEC groups and were significantly more frequent than in controls. These findings confirm that extreme prematurity not only predisposes to NEC but also increases the likelihood of severe disease. Similar observations were reported in large cohort and metaanalysis studies showing that infants born before 28 weeks have up to a fourfold increased NEC risk and higher disease severity (17).

Low birth weight represents another major risk factor closely linked to prematurity. Previous studies have demonstrated that birth weight below 1.000 g is strongly associated with NEC development due to intestinal immaturity, impaired perfusion reserve, dysbiosis, and fragile mucosal integrity (9,18). In our study, the proportion of infants with birth weight <1.000 g was more than twice as high in NEC groups compared with controls. Logistic regression analysis also confirmed that decreasing birth weight markedly increased the odds of both stage II and stage III NEC. These findings are consistent with previous prediction models in very low birth weight infants, which reported AUC values around 0.720-0.83 when gestational age and birth weight were included (15,16). Although our model used fewer parameters and achieved moderate AUC values, it highlights the clinical usefulness of simple and readily available predictors.

Perinatal hypoxia and haemodynamic instability are additional contributors to NEC pathogenesis. Low APGAR scores and the need for resuscitation were significantly more frequent in our stage II and stage III groups. Earlier studies have shown that low APGAR scores reflect perinatal hypoxia and are associated with NEC and other neonatal morbidities (8,10). Hypoxia leads to mesenteric vasoconstriction and intestinal ischaemia, initiating mucosal injury and inflammatory activation (7). Our findings therefore support the concept that perinatal depression may play a role in triggering NEC in susceptible preterm infants.

The need for intensive respiratory support was also significantly higher in NEC groups. Conditions such as RDS and mechanical ventilation may impair systemic oxygenation, reduce mesenteric perfusion, and disrupt mucosal integrity, thereby facilitating NEC development (7). These findings are consistent with the pathophysiological model linking systemic hypoxia, intestinal ischaemia, and bacterial translocation.

Umbilical catheterisation was markedly more frequent in NEC patients, particularly in stage III disease. Umbilical arterial catheter use has been reported to impair intestinal microcirculation and prolonged catheter duration has been associated with Bell stage III NEC (11). National guidelines also highlight catheter-related infection and sepsis as potential contributors to NEC (1). In our

analysis, UAC and UVC use were associated with an increased risk of advanced stage NEC. However, this association should be interpreted with caution, as catheter use is more common in smaller, sicker infants who are already at higher risk for NEC. The observed association may reflect the underlying severity of illness rather than a direct causal effect of catheterization. These findings support the need for careful catheter management and minimisation of catheter duration whenever possible.

Patent ductus arteriosus was also more common in NEC groups. Patent ductus arteriosus may alter systemic circulation through left to right shunting, leading to fluctuations in mesenteric perfusion and intestinal hypoxia. Metaanalysis data have shown that haemodynamically significant PDA is associated with increased NEC incidence (12). These findings support the hypothesis that systemic haemodynamic instability contributes to intestinal injury.

Inflammation plays a central role in NEC progression. Interleukin 6 levels were significantly higher in stage III NEC and were also elevated at NICU admission in infants who later developed severe disease. Previous studies have demonstrated that IL6 may help differentiate NEC from late onset sepsis and reflect disease severity (13,19,20). Our results support IL6 as a potential biomarker for NEC risk stratification, although larger studies are needed to determine its predictive accuracy.

Radiological evaluation remains essential for NEC diagnosis. Plain abdominal radiography showed high diagnostic sensitivity in stage II and III NEC, consistent with its established role in clinical practice (4,5). Abdominal ultrasonography was positive more frequently in advanced disease, in line with recent studies demonstrating increasing diagnostic utility of ultrasound with higher NEC stages (14). Combining radiography and ultrasonography may therefore improve early detection.

Taken together, our findings emphasise that NEC results from the interaction of prematurity related intestinal vulnerability, perinatal hypoxia, haemodynamic instability, inflammatory activation, and iatrogenic factors such as catheterisation. These observations are consistent with current NEC pathogenesis models described in recent reviews and clinical studies (5,7).

Limitations

Our study has some limitations. First, the sample size was relatively limited, particularly in the extremely preterm subgroup, which may explain the lack of significance for some gestational age categories. Second, biomarker evaluation was limited to IL6; inclusion of additional inflammatory and oxidative stress markers could improve predictive accuracy. Third, the singlecentre design may limit generalisability. Fourth, the temporal relationship between certain variables (e.g., lateonset sepsis, metabolic acidosis, inotrope use) and NEC onset could not be fully established in this retrospective analysis. Some of these factors may develop secondary to NEC rather than preceding it, introducing potential reverse causation bias. Fifth, the high odds ratios observed in the logistic regression models, particularly for lateonset sepsis in stage II NEC, suggest potential model instability or overfitting due to small subgroup sizes and the limited number of events per variable. These results should be interpreted as exploratory and hypothesis-generating rather than confirmatory. Sixth, although controls were matched for

gestational age and birth weight, residual confounding from unmeasured variables cannot be excluded. Future multicentre studies with machinelearningbased models incorporating clinical, biochemical, and radiological parameters may provide more precise NEC prediction.

Conclusion

In conclusion, prematurity, low birth weight, perinatal hypoxia, haemodynamic instability, catheter exposure, PDA, and elevated IL6 levels were strongly associated with NEC development and severity in our cohort. These findings support the multifactorial pathogenesis of NEC and highlight the importance of early risk identification and preventive strategies in preterm infants. This retrospective study identifies several factors associated with NEC severity and mortality; however, definitive causal relationships cannot be established from these observational data. Early identification of highrisk infants based on gestational age, birth weight, perinatal hypoxia, catheter exposure, PDA, and inflammatory markers may allow targeted preventive strategies such as careful feeding protocols, early PDA management, strict catheter policies, and close monitoring. Incorporation of simple clinical predictors into risk models may be especially valuable in resource-limited NICU settings. Prospective multicentre studies are warranted to validate these findings and to better define the temporal relationships between potential risk factors and NEC development.

Ethics committee approval

This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Ankara Bilkent City Hospital (05.03.2025, reference number: TABED2/1011/2025).

Contribution of the authors

Study conception and design: NDİ,CT; data collection: NDİ,YEİ; analysis and interpretation of results: NDİ,CT; draft manuscript preparation: NDİ, YEİ. All authors reviewed the results

and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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