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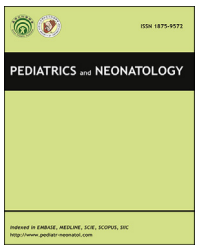

ORIGINAL ARTICLE

Maternal and Placental Risk Factors for Developing Necrotizing Enterocolitis in Very Preterm Infants

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Key Words
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preterm

Background: Despite the clinical relevance of necrotizing enterocolitis (NEC), it remains difficult to predict which preterm infants are more likely to develop NEC. Contrary to the neonatal risk factors for the development of NEC, little information is available regarding maternal (prenatal) risk factors. We aimed to identify maternal risk factors associated with the subsequent development of NEC in very preterm infants and to determine whether the placental inflammatory lesions were related to the NEC.
Methods: This retrospective cohort study examined newborns born at < 32 weeks ($n = 354$) between July 2003 and July 2014 at a university teaching hospital. Medical records of eligible newborns and their mothers were reviewed. Maternal blood white blood cell and differential counts were measured at admission and the placentas were examined histologically after delivery. The primary outcome measure was NEC Bell Stage $\geq$ IIa. Bivariate analyses and multivariate logistic regression were used for the statistical analyses.
Results: NEC was diagnosed in 26 of 354 very preterm infants (7.3%), including 19 Stage II and seven Stage III infants. Multivariate regression analysis identified maternal neutrophil-to-lymphocyte ratio [odds ratio (OR) = 1.08, $p = 0.002$], multiparity (OR = 3.41, $p = 0.013$), and birth weight (OR = 0.07 per kg increase, $p = 0.01$), but not clinical and histological chorioamnionitis and funisitis as significant predictors of NEC.
Conclusion: Maternal neutrophil-to-lymphocyte ratio, parity, and birth weight can independently predict the risk of NEC in very preterm infants, whereas clinical and histological chorioamnionitis and funisitis are not predictive of NEC.

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1. Introduction

Necrotizing enterocolitis (NEC), which affects 11–15% of very low birth weight infants, is one of the most devastating gastrointestinal emergencies encountered during the intensive care of preterm infants.[1–3] Despite the clinical relevance of NEC, it remains difficult to predict which preterm infants are more likely to develop NEC, leading to considerable morbidity and death with a mortality rate of up to 30%.[2,3] Therefore, it is important to identify infants at the highest risk for NEC in a timely manner because preventive measures for NEC (e.g., single course of antenatal corticosteroids, human milk feeding, standardized feeding guidelines, and probiotics) can be adopted to potentially reduce this risk.[2]

Although it is generally accepted that prematurity, low birth weight, formula feeding, and neonatal infection are neonatal risk factors for the development of NEC,[1–5] little information is available regarding maternal (prenatal) risk factors. In this respect, subclinical chorioamnionitis is reportedly associated with preterm birth (the main identifiable cause of NEC), the developmental changes of several fetal organs including the gut, and a higher incidence of neonatal morbidity, especially in infants whose placentas show fetal inflammation.[6,7] Other prenatal risk factors associated with the development of NEC in previous studies were maternal hypertensive disease, maternal infection, maternal exposure to antibiotics and steroids, and conditions adversely affecting placental blood flow.[1–3,5,8–11] However, regarding these numerous maternal and placental risk factors for NEC, previous reports have been somewhat conflicting.[1,3,5,8,10] To clarify this issue, we aimed to identify maternal risk factors associated with the subsequent development of NEC in very preterm infants born before 32 weeks gestation and to determine whether the placental inflammatory lesions were related to the NEC.

2. Methods

This retrospective cohort study was conducted at Seoul National University Bundang Hospital, Seongnam, Korea, from July 2003 to July 2014. All live singleton infants born between 23^{+0} weeks and 31^{+6} weeks and admitted to a neonatal intensive care unit during this period were identified. The inclusion criteria were as follows: (1) born live; (2) singleton; (3) born at $<$ 32 weeks gestation and surviving the first 15 days of life; (4) infants whose placentas underwent histopathological examination; (5) infants whose mothers had differential white blood cell (WBC) counts taken on admission; and (6) umbilical artery acid-base status measured immediately after delivery. Exclusion criteria included (1) major congenital anomalies, (2) twin or higher-order multiple births, (3) infants with isolated spontaneous intestinal perforation, and (4) outborn infants.

For all infants who were delivered preterm at our institution, the placentas were sent for histopathological examination as part of routine clinical practice, and acid-base status measurements in the umbilical artery were routinely performed at the time of delivery. Gestational age was calculated based on the last menstrual period and confirmed by a first- or second-trimester ultrasound. The Institutional Review Board of Seoul National University Bundang Hospital approved this study (project number B-1006/103-102). Informed consent was waived by the Institutional Review Board.

The following data were extracted from the obstetric and neonatal database: maternal and infantile demographic characteristics, maternal blood WBC and differential count at admission, cause of preterm delivery, antenatal use of medications, mode of delivery, clinical diagnosis of chorioamnionitis, placental histopathology, umbilical artery pH, neonatal blood WBC and differential count within the first 24 hours of life, use of indomethacin or ibuprofen for hemodynamic significant patent ductus arteriosus, use of systemic steroids, mechanical ventilation, red blood cell transfusions, diagnosis of NEC in the first 2 weeks or beyond, proven sepsis, respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), and intraventricular hemorrhage. All suspected NEC cases were reviewed by a single neonatologist who was blinded to the maternal details and placental pathology results.

NEC was diagnosed according to the modified Bell's staging criteria, and infants with Stage IIa or greater were defined as having NEC.[12] RDS, BPD and intraventricular hemorrhage were diagnosed according to the definitions previously described.[13–15] Proven sepsis was identified when causative organisms of systemic inflammation were identified on at least two sets of blood cultures. Histological chorioamnionitis and funisitis were diagnosed according to previously published criteria.[13] Clinical chorioamnionitis was diagnosed according to the criteria of Gibbs et al.[16] Neutrophil-to-lymphocyte ratio (NLR) was calculated as the absolute neutrophil count divided by the absolute lymphocyte count. Multiparity was defined as parity greater than or equal to one prior live birth.

We conducted all analyses using SPSS version 22.0 (IBM SPSS Statistics, Chicago, IL, USA). Continuous data were assessed for normality using the Shapiro–Wilk test and analyzed using Student t test and the Mann–Whitney U test. Categorical data were analyzed using the χ^2 test or Fisher's exact test as appropriate. Continuous data are expressed as mean and standard deviation (SD; for normally distributed variables) or median and interquartile range (for non-normally distributed variables), while categorical data are given as number and percentage. A multiple logistic regression analysis was then used to identify prenatal

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and placental factors that were significantly and independently associated with the development of NEC after controlling for potential postnatal risk factors. Variables with p values < 0.1 on univariate analysis were included in a logistic regression analysis. Potential interactions between independent variables were evaluated. A receiver-operating characteristic curve analysis was conducted for NLR, gestational age at birth, and birth weight to determine the best cut-off values (maximizing the sum of sensitivity and specificity) for NEC. All reported p values are two-sided with a significance level of 0.05.

3. Results

During the study period, of the 426 live singleton infants (23^{+0}–31^{+6} weeks), 354 met eligibility criteria and were included in the final analysis. Infants who died in the delivery room ($n = 17$) or by < 15 days of age ($n = 21$), had major congenital malformations ($n = 7$), or had an incomplete data set [lack of maternal blood WBC on admission ($n = 17$) and lack of placental pathology ($n = 9$)] were excluded. The mean gestational age of the study cohort was 29^{+0} weeks (SD, 2^{+0} weeks; range, 23^{+4}–31^{+6} weeks) and the mean birth weight was 1225 g (SD, 373 g; range, 420–2275 g). Stage II or higher NEC was diagnosed in 26 of 354 infants (7.3%) at some point during their hospital stay, including 19 Stage II and seven Stage III infants. Surgery was performed in 11 (42%), and the overall mortality rate of these infants was 7% ($n = 2$).

Table 1 compares the maternal and obstetric characteristics between neonates with and without NEC. There were no differences in maternal age, preeclampsia, preterm labor, premature rupture of membrane rates,

Table 1 Maternal and obstetric factors in relation to the subsequent development of NEC.

	NEC ($n = 26$)	No NEC ($n = 328$)	p
Maternal age (y)	32.1 ± 3.7	32.4 ± 3.9	0.73
Multiparity	19 (73.1)	155 (47.3)	0.011
Cause of preterm delivery			0.72
Preterm labor	11 (42.3)	122 (37.2)	
PPROM	7 (26.9)	113 (34.5)	
Preeclampsia	6 (23.1)	62 (18.9)	
Others	3 (11.5)	30 (9.1)	
Cesarean delivery	19 (73.1)	205 (62.5)	0.28
Antenatal steroids	21 (80.8)	277 (84.5)	0.58
Antenatal antibiotics	10 (38.5)	168 (51.2)	0.21
Antenatal tocolytics	13 (50.0)	197 (60.1)	0.32
Blood WBC counts ($\times 10^3/mm^3$)	13.05 ± 4.93	12.41 ± 4.35	0.48
Blood NLR, median (IQR)	12.5 (5.0–15.7)	5.9 (3.9–9.6)	0.002
Gestation at admission (wk)	26.3 ± 4.2	28.1 ± 2.6	0.002
Admission to delivery interval (d)	0 (0–4.5)	3 (0–7.0)	0.69
Clinical chorioamnionitis	1 (3.8)	24 (7.3)	> 0.99
Histologic chorioamnionitis	11 (42.3)	164 (50.0)	0.45
Funisitis	3 (11.5)	70 (21.3)	0.23

Data are presented as n (%) or mean ± standard deviation, unless otherwise indicated.
IQR = interquartile range; NEC = necrotizing enterocolitis; NLR = neutrophil–lymphocyte ratio; PPROM = preterm premature rupture of membranes; WBC = white blood cell.

Table 2 Neonatal characteristics in relation to the subsequent development of NEC.

	NEC ($n = 26$)	No NEC ($n = 328$)	p value
Birth weight (g), median (IQR)	970 (780–1180)	1250 (990–1520)	< 0.001
Gestational age at birth (wk)	27.8 ± 1.9	29.1 ± 2.0	0.001
Male gender	9 (34.6)	178 (54.3)	0.18
Apgar score < 7			
1 min	23 (88.5)	260 (79.3)	0.32
5 min	16 (61.5)	134 (40.9)	0.040
Umbilical artery pH	7.29 ± 0.08	7.28 ± 0.07	0.53
Blood WBC counts ($\times 10^3/mm^3$), median (IQR)	7.91 (4.93–13.30) ($n = 24$)	8.70 (5.59–15.35) ($n = 314$)	0.341
Blood NLR, median (IQR)	0.61 (0.28–2.04) ($n = 24$)	0.73 (0.33–1.73) ($n = 314$)	0.662
Postnatal steroids	10 (38.5)	69 (21.0)	0.040
Postnatal indomethacin/ibuprofen	13 (50.0)	108 (32.9)	0.077
Mechanical ventilation	22 (84.6)	225 (68.6)	0.12
Red blood cell transfusion	23 (88.5)	202 (61.6)	0.005
Umbilical catheterization	3 (11.5)	30 (9.1)	0.722
Umbilical arterial catheterization	3 (11.5)	22 (6.7)	0.413
Umbilical venous catheterization	2 (7.7)	25 (7.6)	1.000
Proven sepsis	6 (23.1)	33 (10.2)	0.044
Respiratory distress syndrome	21 (80.8)	204 (62.4)	0.088
Bronchopulmonary dysplasia, at least moderate	9 (34.6)	83 (25.3)	0.55
Intraventricular hemorrhage, Grade ≥ 2	1 (3.8)	29 (8.5)	0.40
Mortality during initial hospitalization	2 (7.7)	12 (3.7)	0.28
Hospital duration (d), median (IQR)	59.7 (23.4–86.0)	52.5 (41.5–74.5)	0.42

Data are presented as n (%) or mean ± standard deviation, unless otherwise indicated.
IQR = interquartile range; NEC = necrotizing enterocolitis; NLR = neutrophil–lymphocyte ratio; WBC = white blood cell.

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cesarean delivery rate, prevalence of clinical and histologic chorioamnionitis, funisitis, and rates of antenatal tocolytics, steroids, and antibiotics treatment. However, the group of mothers delivering neonates who developed NEC had a significantly higher proportion of multiparity and higher median blood NLR at admission and they were admitted to the hospital at a significantly earlier gestational age.

Table 2 shows neonatal characteristics and morbidity in relation to the subsequent development of NEC. Neonates who developed NEC were more likely to be of lower birth weight and gestational age, receive systemic steroids and red blood cell transfusion, and have higher rates of a low Apgar score at 5 minutes and culture-proven sepsis. The use of indomethacin/ibuprofen and RDS had a borderline association with the development of NEC ($p = 0.077$ and $p = 0.088$, respectively).

The multivariable logistic regression analysis results are shown in Table 3. Maternal blood NLR, parity, and birth weight were the only variables statistically significantly associated with the subsequent development of NEC. The receiver-operating characteristic curves for NLR, birth weight, and gestational age at birth predicting NEC were above the 45° line, indicating a significant relationship between these parameters and NEC (Figure 1). The best cutoff values (sensitivity, specificity) for predicting NEC were 8.35 for maternal NLR (61.5%, 68.3%), 1095 g for birth weight (73.1%, 66.5%), and 28^{+9} weeks for gestational age (73.1%, 60.7%).

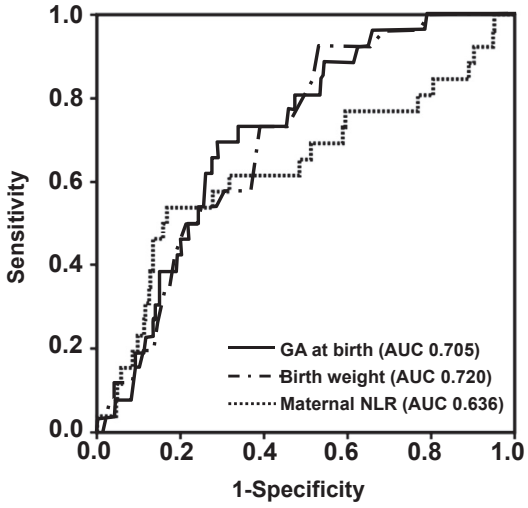

Figure 1 Receiver operating characteristics curves for GA at birth, birth weight, and maternal NLR in predicting necrotizing enterocolitis (GA: AUC 0.705, SE 0.044, $p < 0.001$; birth weight: AUC 0.720, SE 0.043, $p < 0.001$; maternal NLR: AUC 0.636, SE 0.065, $p = 0.020$; respectively). AUC = area under the curve; GA = gestational age; NLR = neutrophil–lymphocyte ratio; SE = standard error.

4. Discussion

We demonstrated that elevated maternal NLR at the time of admission and multiparity was associated with the occurrence of NEC. This association was maintained after the adjustment for known postnatal risk factors, including gestational age at birth, birth weight, proven sepsis, red blood cell transfusion, and postnatal use of steroids. By contrast, neither histologically confirmed chorioamnionitis nor funisitis was associated with NEC. These observations suggest that maternal systemic inflammation, as indicated by elevated NLR, may be implicated in the pathophysiological mechanism of NEC development irrespective of the presence of a localized intrauterine infection/inflammation (reflected by histological evidence of inflammation in the placental tissue).

Table 3 Risk factors associated with the subsequent development of NEC according to logistic regression analysis.

Risk factors	OR	95% CI	*p*
Maternal blood NLR	1.08	1.03–1.14	0.002
Multiparity	3.41	1.30–8.96	0.013
Birth weight (kg)	0.07	0.01–0.53	0.010
Gestational age at birth (wk)	1.14	0.85–1.54	0.38
Apgar score at 5 min < 7	1.26	0.48–3.32	0.64
Respiratory distress syndrome	1.52	0.43–5.36	0.52
Postnatal steroids	0.72	0.24–2.20	0.57
Postnatal indomethacin/ibuprofen	1.21	0.49–3.0	0.68
Proven sepsis	1.41	0.42–4.67	0.58
Red blood cell transfusion	1.86	0.45–7.70	0.39

CI = confidence interval; NLR = neutrophil–lymphocyte ratio; OR = odds ratio.

The role of postnatal systemic infection/inflammation, including sepsis, in the pathogenesis of NEC is well recognized.[1,4,17] However, the contribution of infection/inflammation in prenatal period, such as histological and clinical chorioamnionitis and intra-amniotic infection and/or inflammation, is not clear. With respect to the association between histological chorioamnionitis and NEC, several studies have shown mixed results; some studies reported no associations, whereas other studies reported an association that reached a high level of statistical significance in the chorioamnionitis with fetal involvement (i.e., funisitis) category.[10] Our findings were in line with the results of previous studies and a recent meta-analysis conducted by Been et al.[10] In contrast to the recent meta-analysis,[10] we found that neither clinical chorioamnionitis nor funisitis was associated with increased incidence of NEC. Given that the incidence of NEC and chorioamnionitis has been reported to vary substantially among studies and NEC has a multifactorial pathogenesis,[2,10] this discrepancy may be derived from the differences in inclusion criteria, diagnostic criteria for both entities, standard care in the mother and neonatal intensive care units, sample size, and whether adjustments were made for known risk/protective factors. Similarly, our results about the lack of an association between preeclampsia and NEC were comparable to some previous findings[5] but differed from others.[8,9] The same explanation can be applied to differences in results.

A unique finding of this study was that elevated maternal NLR on admission was independently associated with NEC development, even after adjustment for birth weight,

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gestational age, and other confounders. To the best of our knowledge, this is the first report to describe maternal NLR in relation to the postnatal development of NEC. Indeed, blood NLR is a useful diagnostic and prognostic marker in disease states with low-grade insidious systemic inflammation (leading to atherosclerosis), including diabetes, obesity, metabolic syndrome, hypertension, and cardiovascular disease.[18,19] Moreover, recent studies have shown a significant association between high NLR and endothelial dysfunction,[19,20] leading to vascular dysfunction and atherosclerosis. Therefore, our data show that the association between high NLR and NEC may be because the measurement of blood NLR could potentially be affected by factors that initiate placental vascular dysfunction, which may affect fetuses, facilitating fetal circulatory adaptation to hypoxia and resulting in intestinal ischemia being a predisposing factor for NEC. In support of this view, Ogunyemi et al[21] found that vascular and coagulation placental findings increased the risk of NEC. Another plausible explanation may be that blood NLR is elevated in the localized intrauterine infection and/or maternal systemic infection (like exposure to prenatal lipopolysaccharide), which may result in the fetal gut inflammation and injury mediated by proinflammatory cytokines and dysregulation of inducible nitric oxide synthase as confirmed in animal studies.[22,23]

A particularly interesting finding in our study was that multiparity was significantly and independently associated with an increased risk of NEC development. We cannot explicitly explain this finding, but the most plausible explanation based on previous findings is that the exposure of the mothers to stress factors by repetitive pregnancies, passive transfer of maternally derived antibodies or reactive oxidative derivatives, and gut microbial ecology in offspring might be affected by maternal parity.[24–26] Indeed, a recent study demonstrated that oxidative stress indicators in cord blood, which is known to be useful for identifying infants at risk for NEC,[25] were significantly higher in newborns of multiparous women compared to those of primiparous women.[27] Regrettably, contrary to the animal study done by Carney-Hinkle et al,[26] there has been no study in human infants regarding the relationship between the passive transfer of immunomodulating molecules and change of gut microbial ecology in neonates and maternal parity; therefore, further research is required to clarify this issue. Similar to NEC, in the setting of neonatal respiratory risk such as RDS and BPD, Papadakis et al[28] also showed that multiparity was an important risk factor in infants born to women with preterm premature rupture of the membranes.

In the present study, the incidence of NEC (7.3%) in very low birth weight infants was similar to previous findings.[8,29] Our postnatal findings are also similar to those of the previous reports: NEC was associated with more severe prematurity, lower birth weight, more use of postnatal steroids and red blood cell transfusion, and a greater incidence of sepsis.[1,4,29] Contrary to maternal NLR, neonatal NLR as measured within 24 hours of birth was not associated with NEC development. This finding is consistent with data in the setting of retinopathy of prematurity study demonstrating that NLR measured in the first 24 hours was not an independent predictor of retinopathy of prematurity development.[30] Indeed, our finding is not unexpected because elevated NRL within 24 hours of birth may reflect *in utero* exposure to infection/inflammation (e.g., histological chorioamnionitis, funisitis, and intra-amniotic infection), but not delayed postnatal exposure to infection/inflammation that is associated with an increased risk for NEC.[4,17]

There were several limitations to the current study. First, its design was retrospective, although the data on prenatal and placental risk factors and pregnancy outcomes were collected prospectively. Second, we included participants over an 11-year period to collect a large number of cases despite advances in the management of NEC in preterm infants during this period. This may have affected the impact of various risk factors on NEC development. Third, the current study was based on data from a single institution; therefore, the generalizability of our observations may be limited. Fourth, we did not correct for a potentially important confounder (i.e., enteral formula feeding).[1,2] The strengths of this study were its use of prespecified consensus criteria for clinical and histological chorioamnionitis and its relatively large sample size.

In conclusion, maternal NLR, parity, and birth weight can independently predict the risk of NEC in very preterm infants, whereas clinical and histological chorioamnionitis and funisitis are not predictive of NEC. Further studies are needed to elucidate the underlying mechanisms by which these prenatal factors such as elevated maternal NLR and multiparity may affect the postnatal development of NEC and to evaluate the efficacy of potential prenatal risk-factor-based interventions to prevent the development of NEC.

Conflicts of interest

The authors report no conflicts of interest.

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