Association of Neutrophil to Lymphocyte Ratio with Preterm Necrotizing Enterocolitis: A Retrospective Case-Control Study

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Research Article

Keywords: diagnostic tools, necrotizing enterocolitis, neutrophil to lymphocyte ratio, predictive cutoff value, preterm neonates

Posted Date: March 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1299492/v1

Abstract

Background

There have been few studies on the relationship between the neutrophil to lymphocyte ratio (NLR) and necrotizing enterocolitis (NEC). We conducted a retrospective case-control study to investigate this relationship in preterm neonates.

Methods

A total of 199 preterm neonates diagnosed with NEC between January 2018 and January 2020 were included in this study. For each preterm infant with NEC that was admitted to the neonatal intensive care unit (NICU), controls were preterm neonates (matched for gestation and year of birth) who were not diagnosed with NEC. Exclusion criteria were post-maturity, small or large for gestational age (week of pregnancy), congenital major anomalies, and cyanotic congenital heart disease. Univariate and multivariate logistic regression analyses were used to identify the association between NLR and preterm NEC.

Results

This study included 93 preterm neonates with NEC and 106 matched controls. There were no significant differences in gestational age (GA), birth weight (BW), age, sex, vaginal delivery (VD), chorioamnionitis (CA), and gestational diabetes mellitus (GDM) between groups. Compared with the control group, the lower and higher NLR levels in the NEC group were statistically different. Following univariate analysis, NLR was a risk factor for NEC (odds ratio [OR], 1.40; 95% confidence interval [CI], 1.00–1.90; $P = 0.042$), and according to multivariate analysis, risk factors for NEC were NLR ≥ 3.20 and NLR < 1.60, within 1 week before NEC diagnosis. Thus, NLR values of ≥ 1.60 and < 3.20 were determined as the predictive cutoff values for protecting preterm infants from NEC (Model □: OR, 0.20; 95% CI, 0.10–0.40; $P < 0.001$) and (Model □: OR, 0.10; 95% CI, 0.00–0.40; $P < 0.001$].

Conclusions

NLR ≥ 1.60 and NLR < 3.20 were associated with a decreased risk of NEC in preterm infants.

Background

Necrotizing enterocolitis (NEC) is the most common and potentially devastating multifactorial life-threatening condition affecting the gastrointestinal tract (GIT) in premature infants and is a leading cause of morbidity and mortality in infants born between 23 and 28 weeks of gestation. It has been reported that NEC prevalence is 5–7% in very low birth weight (VLBW) infants,[1] 20–30% of whom

eventually die.[2] A large collection of the data revealed that 42% mortality was due to NEC in premature infants with birth weights of < 750 g; higher birth weights (range, 1250–1500 g) were associated with a significant decrease in mortality to 16%. Moreover, NEC survivors are at high risk of severe long-term complications, such as short bowel syndrome, growth delay, and neurodevelopmental sequelae, which are associated with a low quality of future individual life and increasing long-term social health care costs.[3]

NEC is a multifactorial disease in which the exact pathogenesis remains elusive. Hypoperfusion of the intestines, immaturity of the intestinal barrier system, selection and colonization of harmful bacteria in the gut, bacterial translocation, infection, and inflammatory response contribute to the disease.

The Bell's staging criteria for NEC were first devised in 1978 and modified by Walsh and Kliegman in 1986[4]; almost all neonatologists and medical journals use the modified version. The Bell's staging criteria include three classical NEC stages: mild (Bell's Stage I), moderate (Bell's Stage II), and severe (Bell's Stage III).[5] Mild or suspected NEC (Bell's Stage I) comprises mild systemic symptoms such as temperature instability, bradycardia, and mild nonspecific intestinal symptoms such as mild abdominal distension and occult blood in the stool.[5–7] Since these nonspecific symptoms are often observed in extremely low birth weight infants in the neonatal intensive care unit (NICU), it is very difficult to distinguish between NEC and feeding intolerance, as well as other gastrointestinal diseases and sepsis. Moderate or definitive NEC (Bell's Stage II) further includes radiological findings of pneumatosis of the intestinal wall and/or portal venous gas with moderate systemic signs such as abdominal tenderness, thrombocytopenia, and metabolic acidosis.[5–7] Finally, if there is perforation, the pneumoperitoneum on an X-ray may be found.[8,9] Advanced NEC (Bell's Stage III) that requires a surgical intervention is characterized by a bowel perforation with combined pneumoperitoneum, hypotension, signs of peritonitis, and severe metabolic acidosis.[5–7] However, because of the inter-observer variability, radiological findings cannot accurately predict NEC diagnosis.[10] While the early clinical signs of NEC are usually very discrete and non-specific, the use of Bell's criteria based solely on clinical and radiographic features has significant limitations.[11] Considering these limitations for early NEC diagnosis, research has focused on the discovery of biomarkers capable of prediction, early diagnosis, and discrimination of NEC from other intestinal diseases.

The severity of NEC differs, ranging from mild involvement that would be managed by nothing but fasting for bowel rest or just using antibiotics, to severe intestinal necrosis requiring surgical treatment. Because of the severity of NEC, most physicians may choose to treat it too aggressively. Despite its clear benefits, antibiotic therapy has associated risks. Although the aggressive treatment has the potential to be life-saving, the non-selective, overzealous use of antibiotics could increase the risk of NEC through disruption of the intestinal microbiome. At the same time, prolonged antibiotic usage may result in the evolution of the organisms to a resistant variety, thus, making it more difficult to effectively manage NEC in the future.[12,13]

Since intestinal inflammation of NEC cannot generally be controlled by either conservative or surgical treatment satisfactorily, efforts should be focused on the importance of preventing NEC and improving NEC diagnostic capabilities. To find a diagnostic method with high specificity and sensitivity to identify preterm NEC earlier is an immediate priority.

Therefore, we tried finding specific biomarkers associated with these conditions to improve the NEC diagnostic capabilities. In this context, a biomarker could be defined as any measurable parameter that provides meaningful information regarding the diagnosis of NEC.[14] During the neonatal period, noninvasive and easy-to-use biomarkers that can accurately determine NEC are limited. In the present clinical practice, biomarkers currently include acute phase proteins, inflammatory mediators, and immunoreactive molecules. However, the early prediction and diagnosis of NEC, or the ability to discriminate different stages of NEC correctly from other gastrointestinal diseases, remains unresolved.[3] To our knowledge, there was no single biomarker or cluster of biomarkers that meet the neonatologists' satisfaction. In some recent studies, researchers have focused on noninvasive approaches.[15] Neutrophils are an essential factor in the innate immune response during inflammation. Increased neutrophil levels comprise an appropriate inflammatory response in patients with mild-to-moderately severe disease. One study investigated the incidence of neutropenia (neutrophil counts of $\leq$ 1000 cells/mL) in small for gestational age (SGA) neonates and found that newborn infants have a four-fold increased risk of developing NEC.[16] However, neutrophils may express excessive inflammatory cytokines that contribute to excessive inflammation and tissue damage.

Lymphocytes are also involved in the immune response against bacterial and viral infections.[17] During inflammation, the number of neutrophils increases or decreases, while lymphocytes decrease. The neutrophil to lymphocyte ratio (NLR) reflects changes in neutrophil and lymphocyte levels, which is an indication of inflammation. Therefore, the combination of neutrophil and lymphocyte concentrations indicated as NLR may be more valuable as a marker of inflammation than neutrophilia, neutropenia, or lymphocytopenia alone for predicting bacterial infections. Moreover, NLR has been used as a reliable inflammatory marker and prognostic index for a variety of medical conditions, including ischemic stroke, cerebral hemorrhage, major adverse cardiac events, and solid tumors.[18] A study found that the maternal NLR can independently predict the risk of NEC in very preterm infants.[19]

This study aimed to investigate the relationship between NLR and NEC in preterm neonates. We hypothesized that NLR was associated with an increased risk of NEC based on analysis of existing clinical data.

Methods

Study population

The study population consisted of preterm infants who developed NEC over 2 years from January 2018 to January 2020 at the NICU of the West China Second University Hospital, Sichuan University. Preterm

infants diagnosed with NEC who showed perforations (4 infants), admission age of ≥ 24 h (4 infants), duration of hospital stay < 7 days (9 infants), and with congenital malformations (3 infants) were excluded. The case group included 93 preterm infants who met the criteria for NEC; the control group included 106 preterm infants matched for gestational age and year of birth (Fig. 1).

Study design

We performed a retrospective case-control study. For this analysis, NEC diagnosis was made based on the presence of clinical, radiological, and/or histopathological evidence that fulfilled the Bell's modified criteria. The clinical data, including data pertaining to the demographical characteristics and comorbidities of the mother, were collected from the patients' electronic medical records. NLR was determined using the mean neutrophil and lymphocyte counts of all blood tests within 1 week before NEC diagnosis. Initial laboratory investigations included the white blood cell (WBC) count, platelet (PLT) count, and C-reactive protein (CRP) levels.

Statistical analysis

For descriptive analyses, categorical variables and continuous variables were described as percentages and mean (standard deviation), respectively. The distribution of each covariate of the exposed and non-exposed groups was compared using the t-test (normal distribution) or Kruskal–Wallis rank sum test (non-normal distribution) for continuous variables and the chi-square test for categorical data (Table 1). Next, univariate logistic regression (Table 2) and multivariate logistic regression models (Table 3) were used to examine whether the NLR was associated with NEC in preterm neonates. Statistical results were displayed as odds ratio (OR) with their corresponding 95% confidence interval (CI). All analyses were performed using R (http://www.R-project.org) and Empowerstats software (www.empowerstats.com, X & Y Solutions, Inc.)

Table 1
Baseline characteristics of the study participants (N = 199)

NEC	Case group (n = 93)	Control group (n = 106)	Standardize diff.	*P*-value
Gestational age, weeks (Mean ± SD)	32.74 ± 2.52	32.46 ± 2.75	0.10 (-0.17-0.38)	0.466
Birth weight, g (Mean ± SD)	1733.83 ± 504.75	1793.36 ± 581.09	0.11 (-0.17-0.39)	0.444
Age, hours (Median[Q1-Q3])	0.42 (0.35–0.87)	0.48 (0.36–0.94)	0.00 (-0.28-0.28)	0.993
SEX (n, %)			0.18 (-0.10-0.46)	0.209
Male	43 (46.24%)	59 (55.14%)		
Female	50 (53.76%)	47 (44.86%)		
NLR (Mean ± SD)	2.10 ± 1.40	1.70 ± 0.50	0.40(0.10–0.70)	0.008
CRP, mg/L, (Median[Q1-Q3])	4.89 (1.26-18.00)	3.30 (2.33–6.13)	0.48 (0.20–0.76)	< 0.001
VD (n, %)			0.08 (-0.20-0.36)	0.577
No	71 (76.34%)	78 (72.90%)		
Yes	22 (23.66%)	28 (27.10%)		
CA (n, %)			0.02 (-0.26-0.29)	0.907
No	54 (58.06%)	63 (58.88%)		
Yes	39 (41.94%)	43 (41.12%)		
PE (n, %)			0.38 (0.10–0.66)	0.008
No	40 (43.01%)	66 (61.68%)		
Yes	53 (56.99%)	40 (38.32%)		
GDM (n, %)			0.27 (-0.01-0.55)	0.061
No	46 (49.46%)	67 (62.62%)		
Yes	47 (50.54%)	39 (37.38%)		

For explanation of abbreviations, see the main text

Table 2
Univariate analysis for necrotizing enterocolitis

	Statistics	OR (95% CI)	*P*-value
NLR	1.90 ± 1.10	1.40 (1.00-1.90)	0.042
NLR tripartite group			
Low	66 (33.16%)	1.00	
Middle	66 (33.16%)	0.00 (0.00-0.10)	< 0.001
High	67 (33.68%)	0.90 (0.40-2.00)	0.756
NLR threshold value group			
<1.60	97 (48.70%)	1.00	
≥1.60, < 3.20	71 (35.70%)	1.60 (0.52–4.93)	0.413
≥3.20	31 (15.60%)	9.00 (1.65–49.14)	0.011
Gestational age, weeks (Mean ± SD)	32.59 ± 2.64	1.04 (0.94–1.16)	0.464
Birth weight, g (Mean ± SD)	1765.68 ± 546.38	1.00 (1.00–1.00)	0.442
CRP (n, %)	8.86 ± 13.72	1.05 (1.02–1.08)	0.002
CA (n, %)			
No	117 (58.79%)	1.00	
Yes	82 (41.21%)	1.03 (0.59–1.82)	0.907
PE (n, %)			
No	106 (53.27%)	1.00	
Yes	93 (46.73%)	2.13 (1.21–3.76)	0.009
GDM (n, %)			
No	113 (56.78%)	1.00	
Yes	86 (43.21%)	1.71 (0.97–3.01)	0.062
For explanation of abbreviations, see the main text			

Table 3
Relationship between the neutrophil to lymphocyte ratio and necrotizing enterocolitis according to different models

Variable	Crude Model[a]		Model Ⅰ[b]		Model Ⅱ[c]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
NLR	1.40 (1.10–1.90)	0.010	1.70 (1.20–2.40)	0.003	1.60 (1.10–2.40)	0.013
NLR value group						
< 1.60	1.00		1.00		1.00	
≥ 1.60, < 3.20	0.30 (0.10–0.50)	< 0.001	0.20 (0.10–0.40),	< 0.001	0.10 (0.00-0.40)	< 0.001
≥ 3.20	1.00		1.00		1.00	
For explanation of abbreviations, see the main text						
[a]Crude Model not adjusted.						
[b]Model I adjusted for GA, BW, VD.						
[c]Model II adjusted for GA, BW, sex, VD, CA, PE, GDM, and CRP.						

Results

Baseline characteristics

This study was conducted with 199 preterm neonates that were appropriate for gestational age (AGA). Of these, 93 were diagnosed with preterm NEC, and 106 were preterm infants matched for gestational age and year of birth. The general characteristics of the study population are summarized in Table 1. Overall, infants who were diagnosed with NEC had a higher NLR value (2.10 ± 1.40 vs. 1.70 ± 0.50; $p = 0.008$) and CRP value (4.89 [1.26–18.00] vs. 3.30 [2.33–6.13] mg/L; $p < 0.001$). In addition, preeclampsia (PE) was more common in the NEC group (53/93 vs. 40/106; $p = 0.008$). Apart from these three factors, there was no noticeable difference between the two groups regarding gestational age (GA) (32.74 ± 2.52 vs. 32.46 ± 2.75 weeks; $p = 0.466$), birth weight (BW) (1733.83 ± 504.75 vs. 1793.36 ± 581.09 g; $p = 0.444$), age (0.42 [0.35–0.87] vs. 0.48 [0.36–0.94] hours; $p = 0.993$], sex (male: female, 43:50 vs. 59:47; $p = 0.209$), VD (22/93 vs. 28/106; $p = 0.577$), CA (39/93 vs. 43/106; $p = 0.907$), and gestational diabetes mellitus (GDM) (47/93 vs. 39/106; $p = 0.061$).

Association of NLR levels with NEC

To investigate the association of NLR levels with NEC, subjects were divided into three groups according to NLR tertiles. Univariate analysis showed that the NLR was significantly correlated with preterm NEC (odds ratio [OR], 1.40; 95% confidence interval [CI], 1.00–1.90; $P = 0.042$). In addition, the CRP value (OR, 1.05; 95% CI, 1.02–1.08; $P = 0.002$) and PE (OR, 2.13; 95% CI, 1.21–3.76; $P = 0.009$) might also be associated with preterm NEC (Table 2).

After multivariable risk adjustment for potential confounding factors (Table 3), including GA, BW, sex, and VD in Model I and GA, BW, sex, VD, CA, PE, GDM, and CRP in Model II, the NLR was still positively associated with NEC in preterm neonates. In addition, an NLR of ≥ 1.60 and an NLR of < 3.20 within 1 week before NEC diagnosis could significantly decrease the risk of preterm NEC (Model I: OR, 0.20; 95% CI, 0.10–0.40, $P < 0.001$) and (Model II: OR, 0.10; 95% CI, 0.00–0.40; $P < 0.001$). Therefore, NLR values of ≥ 1.60 and < 3.20 were determined as the predictive cutoff values for the preterm NEC group. A threshold, nonlinear association between NLR and NEC was observed in a generalized additive model (GAM) (Fig. 2).

Discussion

The incidence of NEC is extremely high in preterm infants,[20] and is associated with an increase in mortality. Most of the survivors often experience a variety of serious short-term and long-term complications, such as intestinal stenosis, short bowel syndrome, and neurological sequelae.[21,22] Although the literature is limited, the direct hospital cost of NEC has been estimated to be anywhere from 1.4 to over 10 times higher in very low birth weight (VLBW) infants with NEC than in VLBW infants without NEC. The increased cost stems from longer hospital stays and additional medical interventions (e.g., surgery, central line placement, and increased total parenteral nutrition time), as well as the increased risk of morbidities associated with NEC. NEC has a significant negative impact on healthcare utilization and costs. Therefore, a method to reduce the NEC rate will not only prevent the associated mortality and morbidity and improve the neonatal prognosis, but also reduce healthcare and social costs.

The uncertainty in the course of NEC is due to the absence of a definitive etiology and pathogenesis; moreover, it manifests in a variety of ways. The signs and symptoms of NEC may be concealed and nonspecific, making it difficult to diagnose preterm neonates with NEC earlier. Due to a deficiency in available diagnostic skills and tools, and the accelerated progression of the disease, some infants, particularly the premature infants, do not receive timely treatment. In practice, the diagnosis and treatment of NEC involves duplicate blood testing and abdominal X-ray, the use of broad-spectrum antibiotics, and fasting or decreased enteral feeding. Consequently, many infants may develop secondary anemia, further disturbing the gut microbiome and resulting in retarded growth and development. Thus, it is crucial to develop strategies to identify infants who are less susceptible to NEC to avoid excessive treatment.[23] Furthermore, in order to decrease healthcare utilization and costs which are associated with NEC, identifying preterm infants with NEC accurately and rapidly is very important. For the sake of reducing the burden of NEC in preterm neonates, the prediction and early diagnosis of this catastrophic disease are of utmost necessity.

The current clinical practice to diagnose NEC depends on nonspecific systemic symptoms including inflammation, local abdominal signs, and specific radiographs to determine the presence of gastrointestinal inflammation. However, all of these symptoms are non-specific for NEC, thus, confusing it with the differential diagnosis of other conditions, such as neonatal sepsis, other gastrointestinal diseases, and feeding intolerance. When NEC is suspected, the modified Bell's staging criteria should be applied, which allows rapid clinical decision-making. The features of the Bell's staging criteria represent clinical, laboratory, and radiologic signs, most of which are non-specific and may be less sensitive,[23] and there are numerous shortcomings in the current use of Bell's staging criteria.[6,20] The criteria should not be used as a prognosticating diagnostic tool, but only if NEC had already occurred. The ideal diagnostic biomarker should be both highly sensitive, so as not to miss potential cases, and specific to avoid over-treating infants who are not likely to progress to NEC. Moreover, it should be reliable and have accurate predictive value. Other useful features include affordability, reproducibility, and availability.[24] Some researchers have investigated biomarkers as possible tools to predict NEC, such as interleukin-6,[25] intestinal fatty acid-binding protein,[26] and serum amyloid A.[27] However, the majority of these are not available for routine laboratory tests performed at most medical institutions because of medical costs and the complex methodology required. On the contrary, complete blood counts are simple, easy, and convenient to determine.

The increase in neutrophil count and decrease in lymphocyte count is a response to microbial infection. The increase in neutrophils results from reduced apoptosis of neutrophils and rapid mobilization of neutrophils from a marginated pool within the bone marrow.[28–30] Neutrophils are important in removing pathogens, but neutrophil infiltration and activation also result in major tissue injury associated with acute and chronic inflammatory disorders.[31] Although neutrophils play a vital role in host defense, they can also cause severe morbidity and mortality. The lymphocyte count decreases due to the migration of activated lymphocytes to inflamed tissues and increased apoptosis of lymphocytes.[29,32] It is an indicator of immunosuppression and plays a role in the septic patients' mortality.[33,34] Zahorec previously introduced the NLR as a simple, rapid, and cost-effective method to determine inflammation in critically ill patients.[35] In addition, a previous study showed that this ratio could be utilized as a predictor of disease severity in adult patients.[36] Recent studies found that NLR had a higher sensitivity and specificity for infectious diseases diagnosis.[37,38] For example, Sen et al. showed that NLR preoperatively could be a promising predictor of bacteremia and postoperative sepsis in patients requiring percutaneous nephrolithotomy.[39] In China, there are several similar results, where Yang et al. found that the NLR was significantly higher in the death group than in the control group within 205 adult bloodstream infection patients.[40] In summary, NLR could be utilized to indicate the status of the inflammatory response and the level of physical stress in a timely and accurate manner.[40] In addition, NLR could be used as a predictive marker for patients with infections.

In our study, a statistically significant positive correlation was found between the NLR and preterm NEC when NLR values were ≥ 1.60 and < 3.20. In the univariate analysis, NLR was significantly correlated with

preterm NEC (OR, 1.40; 95% CI, 1.00–1.90; $P = 0.042$). Moreover, CRP and PE may also be associated with preterm NEC. After adjusting for these potential confounders in the multivariate logistic regression analysis, we still found a significant correlation between NLR and preterm NEC. NLR values of ≥ 1.60 and < 3.20 were determined as the predictive cutoff values for the preterm NEC group (OR, 0.20; 95% CI, 0.10–0.40; $P < 0.001$), which is associated with a decreased risk of NEC in preterm infants. In addition, NLR (< 1.60 or ≥ 3.20) may be used as a diagnostic tool for preterm NEC. This ratio may be applied in clinical practice and can be used during routine diagnostic processes for preterm NEC in NICUs.

The present study has some limitations. The inherent bias due to the retrospective design of the study should be mentioned. Moreover, the sample number of this study was small and only from a single center, which may also restrict the accuracy and generalizability of the results.

Conclusions

In conclusion, the NLR, which is an easy, simple, inexpensive, and rapid tool, could be used, in advance, to predict preterm NEC along with other biomarkers. Timely NEC prediction would not only allow for high-risk neonates to receive preventive treatments such as early exposure to the mother's colostrum, careful nutritional consideration, use of probiotics, and increased skin-to-skin care,[41] but also decrease unnecessary antibiotic therapy or even surgical interventions.

Overall, our findings emphasize the necessity to improve medical measures to decrease the incidence of preterm neonates NEC. Future prospective studies with a larger population of preterm infants are required to validate the results of this study. In addition, in the forthcoming studies, we plan to develop a predictive model of the NLR for NEC diagnosis through machine learning, and then prospectively substantiate the model's accuracy using a larger number of preterm infants.

Abbreviations

AGA
appropriate for gestational age
BW
birth weight
CA
chorioamnionitis
CI
confidence interval
CRP
C-reactive protein
GA
gestational age
GIT

gastrointestinal tract
GDM
gestational diabetes mellitus
GAM
generalized additive model
NLR
neutrophil to lymphocyte ratio
NEC
necrotizing enterocolitis
NICU
neonatal intensive care unit
OR
odds ratio
PLT
platelet
PE
preeclampsia
SGA
small for gestational age
VD
vaginal delivery
VLBW
very low birth weight
WBC
white blood cell

Declarations

Ethical approval and consent to participate

The study was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (2020 application 096), and due to the retrospective nature of the data analysis, informed consent from the patient's parents was waivered. All procedures were performed in accordance with relevant guidelines.

Consent for publication

Not applicable.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by the department of Science and Technology of Sichuan Province (2021YJ0171).

Authors' contributions

Hua Wang conceptualized and designed the work, provided feedback about the study design, helped draft the initial manuscript, assisted in the creation of the tables and figures, and critically reviewed and revised the manuscript. Yuju Mu performed the background research and data gathering, assisted in the study methodology, carried out the statistical analyses, developed the figures and tables, reviewed and revised the article, All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Acknowledgments

The authors thank Sichuan University West China Second University Hospital for their assistance with data collection.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

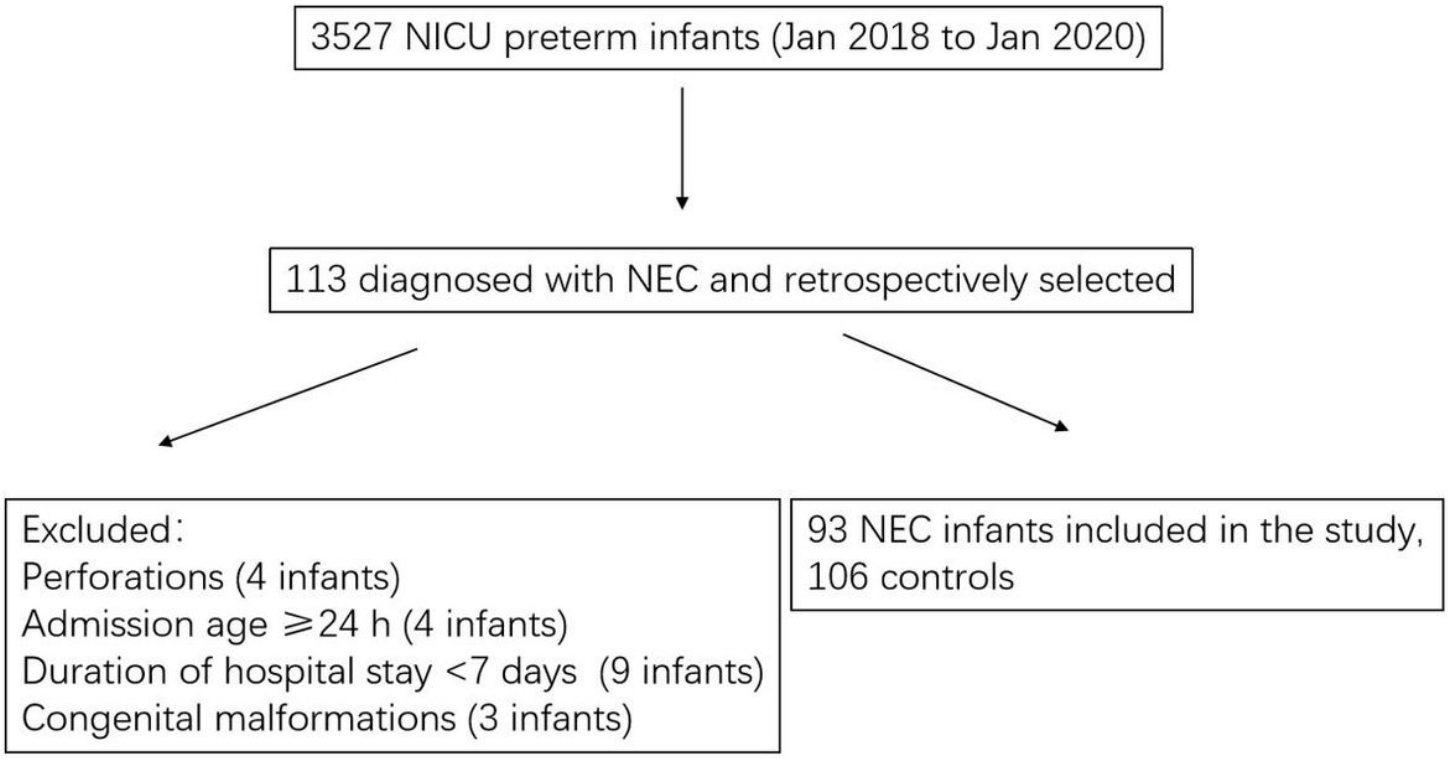

Figure 1

Patients selection flow chart (2018–2020).

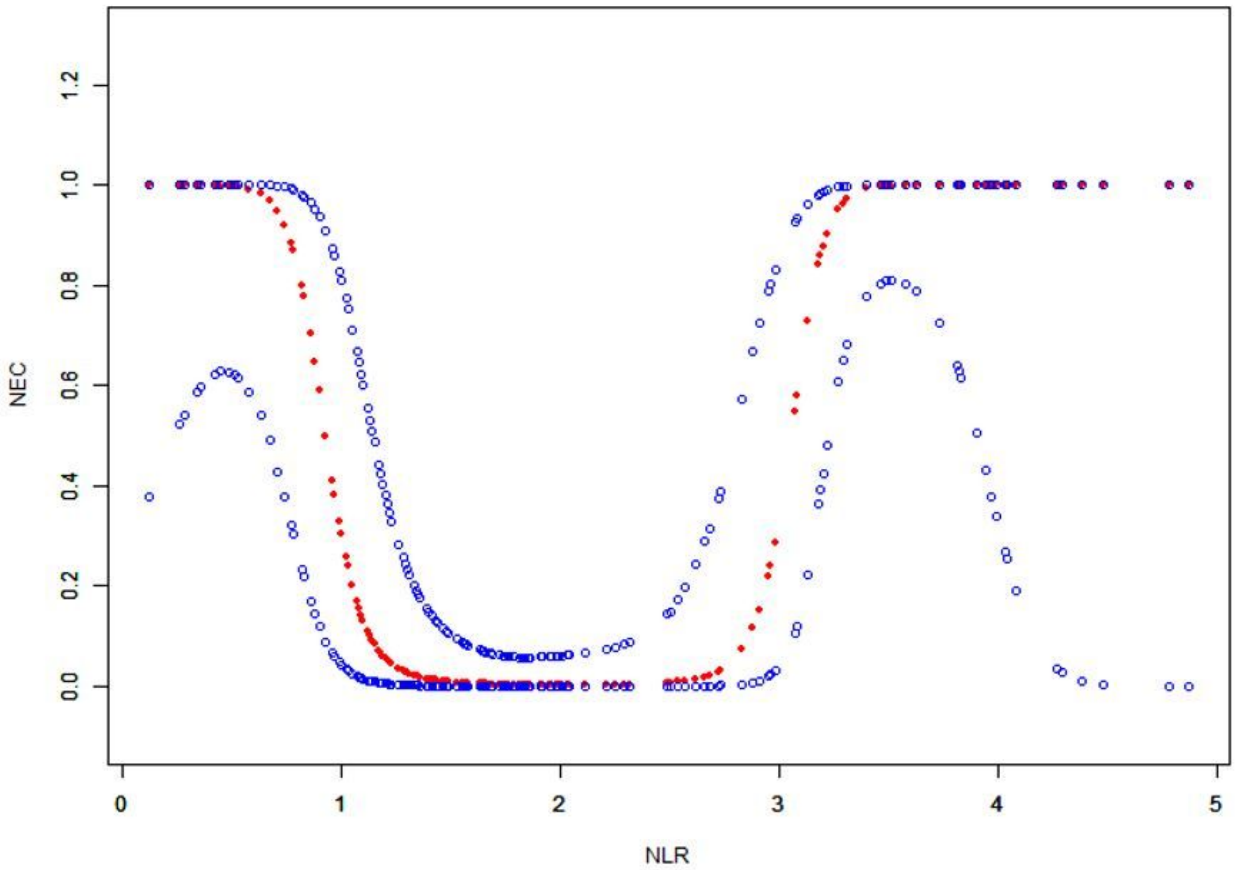

Figure 2

Association between neutrophil to lymphocyte ratio and necrotizing enterocolitis. A threshold, nonlinear association between neutrophil to lymphocyte ratio (NLR) and necrotizing enterocolitis (NEC) was determined ($P<0.05$) from a generalized additive model (GAM). The red line represents the smooth curve fit between variables. The blue bands represent the 95% confidence interval from the fit.