RESEARCH ARTICLE

Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants?

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Background: The neutrophil to lymphocyte ratio (NLR) is an easily accessible biomarker that has been reported to represent disease severity in adult trials. The aim of this study was to evaluate the relationship between culture positiveness and NLR in cases where the reason of sepsis was considered, and to foresight an idea about the active agents.

Methods: Preterm infants with birth weights ≤ 1500 g and/or ≤ 32 gestational weeks were eligible for this study. The postnatal age of all included infants was more than 3 days with clinical and laboratory signs of sepsis. According to the results of blood cultures, all enrolled infants were classified into 2 groups: the culture-proven septic infants and suspected septic infants. The NLR was calculated as the ratio of neutrophil count to lymphocyte count. Initial laboratory investigations included WBC count, platelet count (PLT), C-reactive protein (CRP), and blood cultures.

Results: A total of 127 infants were involved: 57 culture-proven sepsis and 75 suspected sepsis. There were no significant differences between groups regarding gestational age, gender, birth weight, delivery mode, and postnatal age. Receiver operating curve analysis for NLR and CRP was calculated. The area under the curve corresponded to 0.78 ± 0.04 (NLR) vs 0.55 ± 0.05 (CRP). Using a cutoff point of 1.77 for NLR, the sensitivity was 0.73, the specificity was 0.78, and accuracy rate was 0.76.

Discussion: The prediction of NLR, an easy, inexpensive, and rapid method, along with CRP in the neonatal period for diagnosis of sepsis, will be more effective in detecting culture-proven sepsis and in decreasing unnecessary antibiotherapy.

KEYWORDS

late-onset neonatal sepsis, neutrophil to lymphocyte ratio, preterm

1 | INTRODUCTION

Sepsis is a life-threatening condition with significantly high level of mortality both in preterm and term infants. Roughly, it can be defined as a systematic inflammatory response that develops as a result of proven or suspicious infection in neonatal period.¹ Especially in developing countries, early diagnosis and treatment of babies with infections are insufficient. Clinical findings of sepsis in neonates are difficult because many of the symptoms relating to sepsis are obscure and they can be revealed with conditions of other noninfectious diseases. The main point in treatment is an early

diagnosis and appropriate treatment with antibiotics much earlier before the blood culture results are obtained, and the importance of suspicion index based on clinic and laboratory parameters should be emphasized.²

Even though the isolation of microorganisms in blood culture is considered as a golden standard, the results are generally not sufficient due to late and incorrect negative outcomes.³

C-reactive protein (CRP), a major acute phase protein, is a member of pentraxin family and plays a crucial role in the immune system.⁴ Although new biomarkers are being used in various studies, CRP is still the biomarker which is commonly used and worked with.⁵ Around

10–12 h after the infection commences, CRP begins to rise, and it is often used combined with other biomarkers.^{4,5}

In the neonatal period, noninvasive and easily applicable biomarkers with a high level of diagnostic verification are limited. In the new researches being conducted, studies are carried out as relating to noninvasive approaches, which give rise to new hopes for the neonatal period.⁶

Currently, complete blood count (CBC) and mean platelet volume (MPV) values are being used in the diagnosis and monitoring of neonatal sepsis.⁷ In the adult studies, neutrophil/lymphocyte ratio (NLR) has been used in determining the existence and severity of sepsis.^{8,9} This ratio is a sign of inflammation, and it can be used in foreseeing septicemia. The aim of this study was to evaluate the relationship between culture positiveness and NLR in cases where the reason of late-onset neonatal sepsis (LOS) is considered, and to have an idea about the microorganisms in blood. The secondary purpose of our study was that to have an idea about the estimation of possible microorganisms by calculating NLR in culture-positive infants.

2 | PATIENTS AND METHODS

2.1 | Study design and interventions

This study is a prospective observational study that was conducted in the level III neonatal intensive care unit (NICU) at İzmir Tepecik Training and Research Hospital between January 2014 and January 2015. Our NICU unit is an international destination for the treatment of rare and complex medical conditions in neonates. Our hospital has a recognized team of physicians, fellows, surgeons, and nurses who offers excellent state-of-the-art care, and an outstanding record of success in nearly 100 clinical specialties. Each year >10 000 births occurs, and nearly 1500 neonates are admitted to our NICU. Major congenital anomalies, congenital infections, infants born to mothers with clinical chorioamnionitis, or those who had early-onset neonatal sepsis, perinatal asphyxia, or intrauterine growth restriction were excluded from the study. The postnatal age of all included infants was over 3 days with clinical and laboratory signs of sepsis. All patients consecutively admitted to the NICU for suspicion of sepsis were enrolled. The study was approved by the local ethical committee of the hospital (2014/05-2). During the study period, 160 preterm infants whose birth weight was ≤ 1500 g were admitted to NICU, of which 127 infants were considered eligible for the study. The remaining 33 infants were excluded due to the absence of parental consent (10 infants), the presence of congenital heart disease (5 infants), diagnosis of early-onset sepsis/pneumonia (10 infants), symptomatic patent ductus arteriosus required treatment (8 infants). Of the 127 infants studied, 52 infants were classified into the culture-proven sepsis group and 75 into the suspected sepsis group.

2.2 | Definition of sepsis

The diagnosis of sepsis was based on at least 2 clinical and 2 laboratory criteria.⁸ Clinical criteria were as follows: hyperthermia (core body temperature $\geq 38^\circ\text{C}$) or hypothermia $< 36^\circ\text{C}$ or temperature instability, tachycardia (heart rate > 160 beats/min) or bradycardia (heart

rate < 100 beats/min) or rhythm instability, tachypnea (respiratory rate > 60 breaths/min) or recurrent apnea > 20 s, increased oxygen requirements or requirement for ventilator support, feeding intolerance suspected by the presence of gastric residuals, abdominal distension, gross or occult blood in the stool and the onset of crises of apnea/bradycardia, abdominal distension, lethargy and hypotonia, hypotension < 5 th percentile for age or systolic blood pressure < 2 SD below normal of age, and skin and subcutaneous lesions such as mottling and sclerema, whereas laboratory criteria were as follows: white blood cell (WBC) count < 5 or $> 20 \times 10^9$ cells/L, immature to total neutrophil ratio > 0.2 , platelet count (PLT) $< 100 \times 10^9$ /L, and CRP > 10 mg/L. The diagnosis was verified after by positive blood culture.¹⁰

2.3 | Blood sampling and assays

Blood samples and cultures were taken from peripheral veins. Blood samples were taken at the time of initial laboratory evaluation for sepsis. After the blood samples were taken, prophylactic antibiotherapy was started until the results were obtained. Peripheral blood was collected into a tube containing ethylene diamine tetraacetic acid (EDTA) (1.2 mg/mL) for CBC and analyzed using flow cytometry (Beckman LH 780). The NLR was calculated as the ratio of neutrophil count to lymphocyte count.

For blood culture, 1 mL of blood was obtained and placed in 2 culture bottles containing broth to grow aerobic and anaerobic microorganisms. All assays were done in strict accordance with the manufacturers' instructions.

Serum CRP level was analyzed by scattering immunoturbidimetry (Beckman Coulter AU5800). All assays were done in strict accordance with the manufacturers' instructions.

The NLR was determined on a Beckman LH 780 analyzer counting neutrophil and lymphocytes using the WBC differential methods.

2.4 | Group classification

According to the results of blood cultures, all enrolled infants were classified into 2 groups: the culture-proven septic infants and suspected septic one. Initial laboratory investigations included WBC count, PLT, CRP, and blood cultures. The former included neonates with proven (positive blood cultures for microbes or fungi) and suspected sepsis but negative blood cultures.

2.5 | Therapeutic strategy

In our unit, when sepsis was suspected, empirical antibiotic therapy was initiated in these infants pending blood culture results. In our center, the predominant causative organism for LOS in preterm infants is coagulase-negative staphylococci, and the common pathogens are *Staphylococcus aureus* and Gram-negative bacteria. The antibiotic combination consists of vancomycin and aminoglycosides/cephalosporins while awaiting isolation of the causative organism and its antibiotic susceptibility pattern. Antibiotics were re-evaluated when the results of the cultures are available.²

2.6 | Statistical analysis

Statistical data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 18.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were compared using Student's *t* test or Mann-Whitney *U* test. Categorical variables were analyzed using the chi-square test or Fisher's exact test. A *P* value of <.05 was considered statistically significant. Sensitivity, specificity, accuracy rate of clinical findings related with NLR, and CRP were calculated with the following formulas:

$$\text{Sensitivity} = \text{True positives} / (\text{True positives} + \text{False negatives}) \times 100$$

$$\text{Specificity} = \text{True negatives} / (\text{True negatives} + \text{False positives}) \times 100$$

Sensitivity and specificity between the classification and cutoff values were calculated according to the NLR; CRP variables of the groups were examined and expressed by receiver operating curve (ROC) analysis, and the area under the curve (AUC) was calculated for each plot.

3 | RESULTS

Of the total 160 patients with GA ≤ 32 weeks, 33 were excluded from the study due to various reasons, and only 127 patients were included in the trial. The mean gestational age was 28.0 ± 2.5 weeks with the birth weight of 1114.6 ± 314 g and postnatal age of 20.5 ± 15.1 days. The neonates were divided into 2 groups according to the blood culture results: Group I (culture-proven sepsis; $n = 52$; 40.0%) and Group II (suspected sepsis; $n = 75$; 59.0%). There were no significant differences between gestational age, birth weight, gender, delivery method, maternal age, and postnatal age between the 2 groups ($P > .05$). The demographic characteristics of infants in 2 groups are shown in Table 1.

White blood cell and PLT levels were similar in both groups (Table 2). There was a significant increase in NLR in culture-proven septic preterm infants when compared to suspected septic infants (mean 3.69 ± 3.0 vs 1.56 ± 1.83 , $P < .001$) (Table 2). CRP levels did not differ in both groups (Table 2).

Receiver operating curve analysis for NLR and CRP was calculated. The AUC corresponded to NLR 0.78 ± 0.04 vs CRP 0.55 ± 0.05 (95%, confidence interval) (Figure 1). Using a cutoff point of 1.77 for NLR, the sensitivity was 0.73, the specificity was 0.78, and accuracy

TABLE 2 Comparison of laboratory findings

	Culture-proven sepsis ($n = 52$)	Suspected sepsis ($n = 75$)	<i>P</i>
WBC ($\times 10^9/L$) ^a	13.7 ± 7.0	1198 ± 5.2	.09
PLT ($\times 10^9/L$) ^a	261 ± 147	280 ± 154	.47
CRP (mg/L) ^a	30.9 ± 41.5	23.8 ± 25.2	.23
NLR ^a	3.69 ± 3.0	1.56 ± 1.83	<.001

^aAs presented mean $\pm$ SD. $P < .05$ was considered significant (in bold).

rate was 0.76. Using a cutoff point of 7.1 mg/L for CRP, the sensitivity was 0.96, the specificity was 0.24, and accuracy rate was 0.52 (Table 3).

It was found that there was 52 culture-proven septic infants, of which 45 (36%) were Gram-positive and 7 (5%) were Gram-negative. To detect the Gram-positive sepsis of NLR in subgroup analysis, the cutoff value was 1.77, sensitivity was 0.71, and specificity was 0.78 were used. The cutoff value was 2.22, the sensitivity was 0.85, and specificity was 0.82 were used to detect Gram-negative sepsis (Figure 1 and Table 3).

4 | DISCUSSION

In this study, we found that regarding the suspicion for LOS in preterm babies, NLR could predict culture-positive sepsis and that it would be a better indicator than CRP. Furthermore, we found out that in case NLR level increases more, we could have an idea about the pathogenic microorganism.

Neonatal sepsis is an important reason for mortality and morbidity in babies that are admitted in NICU. Each year nearly 400.000 newborn babies lose their lives due to neonatal sepsis, and when the investigated as per countries, it is observed that in developing countries, death incidents relating to neonatal sepsis are much higher.^{11,12} Sepsis ratio in NICUs in Turkey is 9.3%-25%.¹³

Regarding neonatal sepsis diagnosis, blood culture identification, which is accepted as a golden standard, cannot be obtained for all patients, and besides, the results can be obtained only after 24-48 h.¹⁴ Isolation of agent from the culture can vary between 8% and 73%.¹⁵ Due to these reasons, there is often a need for tests that are fast with high level of specificity and sensitivity and that can be used in early diagnosis. When compared with term babies, preterm babies are more inclined to infections. Due to low level of immune responses and due to high level of invasive attempts, LOS risk is higher in preterm babies. As it can lead to mortality and morbidity if it is not diagnosed earlier, for newborn babies, there is need for diagnostic methods which can give quick results.¹⁶ For this purpose, we should list the markers (IL-6, IL-8, CRP, and PCT), which are mostly investigated.¹⁷ Even though there are new markers emerging from various studies, CRP is still considered as the acute phase reactant, which is mostly used. As it is quick, simple, and cheaper compared with other biomarkers, CRP becomes the mostly preferred biomarker in many NICUs. When literature is reviewed,

TABLE 1 Characteristics of the infants

	Culture-proven sepsis ($n = 52$)	Suspected sepsis ($n = 75$)	<i>P</i>
Gestational age (wk) ^a	27.5 ± 2.5	28.3 ± 3.0	.96
Birth weight (g) ^a	1037.8 ± 303	1167.8 ± 313	.54
Male gender	31 (59)	40 (53)	.23
Delivery mode (NVD/C&S)	17/35	39/36	.03
Postnatal age (d) ^a	22.2 ± 16.3	19.2 ± 14.3	.67
Maternal age (y) ^a	28.3 ± 7.4	29.2 ± 5.9	.41

^aAs presented mean $\pm$ SD.

FIGURE 1 ROC analysis of NLR and CRP

TABLE 3 ROC analysis of groups

	Groups	Graphic	Cutoff	Sensitivity	Specificity	Accuracy rate	AUC $\pm$ SE	P value
NLR	Culture-proven/suspected	A	1.77	73.1%	78.7%	76.4%	0.782 ± 0.042	<.001
	Gram (+)/(-)	B	2.14	85.7%	46.7%	21.3%	0.575 ± 0.116	.529
	Gram (-)/suspected	C	2.22	85.7%	82.7%	53.5%	0.822 ± 0.098	.005
	Gram (+)/suspected	D	1.77	71.1%	78.7%	71.7%	0.776 ± 0.044	<.001
CRP	Culture-proven/suspected	E	7.10	96.0%	24.0%	52.8%	0.558 ± 0.056	.273

$P < .05$ was considered significant (in bold).

serum CRP shows different kinetics and cutoff values change within the range of 1.5–20 mg/L, while sensitivity and specificity show variations (74%–98% and 71%–94%, respectively).¹⁸ These differences that are observed in the studies arise due to different kinetics depending on different pregnancy weeks of babies and different body weights. There are arguments regarding the cutoff point of CRP, and it is defended that, especially in incidents of early-onset sepsis, 10 mg/L is more reliable.^{18,19} In the present study, the mean serum CRP was 30.9 ± 41.5 mg/L in culture-proven septic neonates with 96% sensitivity and 24% specificity at a cutoff value of 7.1 mg/L. Nevertheless, NLR is a quick and easy method in detecting culture-proven sepsis as the AUC corresponded to 0.78 ± 0.04 (NLR) vs 0.55 ± 0.05 (CRP).

Complete blood count is a simple and easy that is evaluated in newborn babies routinely in case of infection suspicion. Low white cell

number and high immature/total ratio is the screening method which is mostly used as relating with neonatal sepsis.²⁰ The increase in neutrophils show that a response to microbial infection, and they quickly migrate to the influenced region. At the same time, they play an active role in phagocytosis, release of cytokine, and T-cell activation. On the other hand, many anti-inflammatories become immunosuppressed, while many lymphocytes play a role in apoptosis. For this reason, lymphopenia is an indicator of immunosuppression and it has a role as a reagent for mortality in septic patients.^{21,22} Even though it is stated that there can be a relation among NLR, septic shock, and mortality in adults patients, as they are not stated in the study literature relating to neonatal period, our knowledge about this subject is limited.²² In the study by Omran et al.,²³ NLR values of septic newborn babies and those of healthy babies were compared and it was found out that NLR values were much higher in septic newborn babies. At the same

time, it was observed that with 2.7 cutoff point, the level of sensitivity and specificity were 80% and 57.1%, respectively. In our trial, cutoff point for NLR was 1.77, the sensitivity was 0.73, and the specificity was 0.78. With regard to the differences in cutoff values, we have the opinion that as all the babies in our study are preterm and as their birth weights are lower, specificity levels are higher and cutoff values are lower. There was LOS in all the babies, which were made part of the study at the same time. In the adult studies where the relation between infection and NLR has been investigated, it was stated that this ratio could be a predictor in determining the severity of disease, while it has been used in differentiating bacterial and viral meningitis and in specifying community originated pneumonia.²⁴ When we investigated the relation between babies having identification in blood culture and NLR, we found out that the higher level of NLR could be related to Gram-negative sepsis. However, as number of our cases with Gram-sepsis identification was low, this created a limitation in our study.

As per our knowledge, this is the first study in literature showing that NLR can be used as an important determinant in predicting LOS in preterm babies. It is clearly demonstrated in this study that 1.7 cutoff value is sufficient for predicting culture-positive sepsis. This ratio which can be easily achieved with neutrophil and lymphocyte numbers can be integrated into clinical practices and it can be used in the routine scanning processes for nosocomial septicemia in NICUs.

In conclusion, the prediction of NLR, an easy, simple, inexpensive, and rapid method, along with CRP in the neonatal period for diagnosis of sepsis, will be more effective in detecting culture-proven sepsis and in decreasing unnecessary antibiotherapy. However, these findings still need to be confirmed by other future studies aiming to investigate in large numbers of neonates and other tested markers in diagnosis of neonatal sepsis.

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