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Original Article

Can Neutrophil to Lymphocyte Ratio Predict Duration of Hospitalisation in Term Infants with Early Neonatal Infection?

E ***Guler Kazanci***, EA ***Ozer***, S ***Alkan Ozdemir***

Abstract ***Background:*** Despite improving neonatal care, neonatal infections are still a fundamental problem. ***Objectives:*** This study aims to evaluate the value of the neutrophil-to-lymphocyte ratio (NLR) to predict the duration of hospitalisation in term infants with early neonatal infection. ***Patients and Methods:*** The infants included in the study were divided into two groups: infants who required hospitalisation ≥7 days (increased length of stay (LOS) group) or those who required hospitalisation for less than 7 days (control group). The two groups were compared for gestational age, birth weight, mode of delivery, need for respiratory support, complete blood count parameters, C-reactive protein (CRP), blood culture and chest X-ray. ***Results:*** The study included 120 eligible term newborn infants according to the study protocol. The number of infants needs respiratory support, abnormal chest X-ray and CRP positive cases were statistically significantly higher in the increased LOS group NLR was also statistically significantly higher in the increased LOS group. ***Conclusion:*** This study is the first cohort study that evaluates the value of NLR and CRP for predicting of length of stay in term neonates with early neonatal infection. Our results emphasize that, both NLR and CRP are effective in predicting length of stay in this group.

Key words Lymphocytes; Neutrophils; Newborn; Sepsis

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Introduction

Neonatal infections are a common problem in the neonatal period. It is estimated the prevalence at 1 to 10 per 1000 live births.[1] Early onset neonatal sepsis might show nonspecific signs and symptoms. Therefore, differential diagnosis and predicting prognosis is important. The initial assessment of neonates with sepsis should include complete blood count, C-reactive protein (CRP), blood culture, blood gases and diagnostic image of the lungs. Although the positive blood culture is accepted as a gold standard for the diagnosis of neonatal sepsis, the blood culture results late and has a high rate of false negative outcomes. The neutrophil-to-lymphocyte ratio (NLR) is easy and inexpensive examination. Previous studies have revealed that NLR has a promising sensitivity and specificity in the diagnosis of infections.[2-4] Improving

length of stay prediction is important for neonatal intensive care units settings for resource planning, reducing possibility of healthcare associated infections and improving financial efficiency. The purpose of the study is to evaluate the value of NLR to predicting duration of hospitalisation in term infants with early onset neonatal sepsis.

Methods

This retrospective, single-centre study was conducted at a tertiary neonatal intensive care (NICU) between May 2020 and May 2021. This study was approved by the local ethics committee (2022/3). Infants with a gestational age of ≥37 weeks admitted for signs of early onset neonatal sepsis within 24-hour after birth were enrolled in the study. Patients with perinatal asphyxia, severe congenital abnormalities, inherited metabolic diseases or discharge within the age of 24-hour were excluded.

Data for mode of delivery, birth weight, and gestational age, and gender, duration of oxygen therapy, need of respiratory support and length of hospital stay were recorded from patient files.

Blood cultures and samples were taken on during admission to the neonatal intensive care unit. Peripheral blood was collected into a tube containing ethylene diamine tetra acetic acid (EDTA) (1.2 mg/mL) for complete blood count (CBC) and analysed using flow cytometer (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. C-reactive protein (CRP) level is measured by immunoturbidimetric method. All assays were performed by the manufacturers' instructions.

The infants included in the study were divided into two groups: infants who required hospitalisation ≥7 days (increased length of stay (LOS) group) or those who required hospitalisation for less than 7 days (control group). The two groups were compared for gestational age, birth weight, mode of delivery, need for respiratory support, complete blood count parameters, CRP, blood culture and chest X-ray.

Statistical data were analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were compared using Student's t test or Mann Whitney U test. Categorical variables were analysed using the chi-square test or Fisher's exact test.

A P value of <0.05 was considered statistically significant. Sensitivity, specificity, accuracy rate of clinical findings related with NLR and CRP were calculated with well-known methods. The influencing factors of length of stay were analysed by multivariate logistic regression analysis. Receiver Operating Characteristic (ROC) curves were used to evaluate the diagnostic value of the variables for length of stay. The level of significance was set at a two-sided P value of less than 0.05.

Results

The study included 120 eligible term newborn infants according to the study protocol. The demographic and clinical characteristics of the study group were shown in Table 1. Of them, 58 were hospitalised for less than 7 days. Clinical and laboratory characteristics of the study groups are shown in Table 2. Birth weight was lower in the increased LOS group. The number of infants needs respiratory support, abnormal chest X-ray and CRP positive cases were statistically significantly higher in the increased LOS group. White blood cell and neutrophil counts were statistically significantly higher in the increased LOS group whereas platelet count and platelet indexes were not different. NLR was also statistically significantly higher in the increased LOS group. The sensitivity, specificity, positive and negative predictive values of the NLR and CRP were calculated and compared (Table 3). Despite the sensitivity is higher in NLR than CRP, positive and negative predictive values and specificity are in favour of CRP (Figure 1).

Table 1 Clinical characteristics of the study group

Number of infants	120
Gestational age (week)[a]	37.1 [1.6]
Birth weight (gram)[a]	2846 [505]
Male/Female	74/46
Delivery type (Normal/cesarean section)	25 / 95
Duration of hospitalisation (day)[a]	13.7 [31.2]
Duration of oxygen supplementation (day)[a]	2.27 [2.3]
Number of infants needs respiratory support (%)	73 (60.8)
Number of infants needs antibiotic treatment (%)	35 (29.2)

[a]Data are presented as mean [standard deviation]

Table 2 Clinical and laboratory characteristics of the study groups

	Increased LOS group (n=62)	Control group (n=58)	*p*
Gestational age (week)[a]	36.8 [1.7]	37.5 [1.5]	*0.025*
Birth weight (gram)[a]	2738 [569]	2961 [398]	*0.015*
Male/Female	36/26	38/20	0.40
Delivery type			
(Normal/cesarean section)	14/48	11/47	0.62
Number of infants need respiratory support	55	18	*<0.001*
Number of infants with positive blood culture	8	3	0.20
Number of infants with abnormal chest X-ray	55	8	*<0.001*
Number of infants with positive C-reactive protein	30	5	*<0.001*
Hemoglobin (g/dL)[a]	15.9 [2.8]	17.6 [2.6]	*0.002*
Haematocrit (%)[a]	46.9 [7.8]	51.5 [6.4]	*0.01*
White blood cell (/mm^3)[a]	19049 [4553]	15266 [7072]	*0.002*
Platelet (/mm^3)[a]	237000 [100000]	252000 [62000]	0.31
Platelet mass index[a]	24.5 [11]	26.5 [8.1]	0.32
Mean Platelet Volume[a]	10.06 [3.6]	9.8 [0.9]	0.61
Lymphocyte (/mm^3)[a]	3825 [1842]	5414 [7601]	0.20
Neutrophil (/mm^3)[a]	11968 [4156]	11364 [13438]	*0.023*
Neutrophil/lymphocyte ratio[a]	3.5 [1.3]	2.9 [1.2]	*0.02*

[a]Data are presented as mean [standard deviation]

LOS: Length of stay

Table 3 Comparison of NLR and CRP to predicting length of stay in hospital

	NLR	CRP
Positive predictive value	60.29 (52.23-67.83)	85.7 (71.4-93.5)
Negative predictive value	59.62 (49.17-69.26)	62.3 (56.2-68.10)
Sensitivity	66.13 (52.99-77.67)	48.39 (35.5-61.44)
Specificity	53.45 (39.87-66.66)	91.38 (81.02-97.14)
Area under the curve	0.614	0.687

The numbers in parentheses indicate the 95% confidence interval.

NLR: Neutrophil-to-lymphocyte ratio; CRP: C-reactive protein

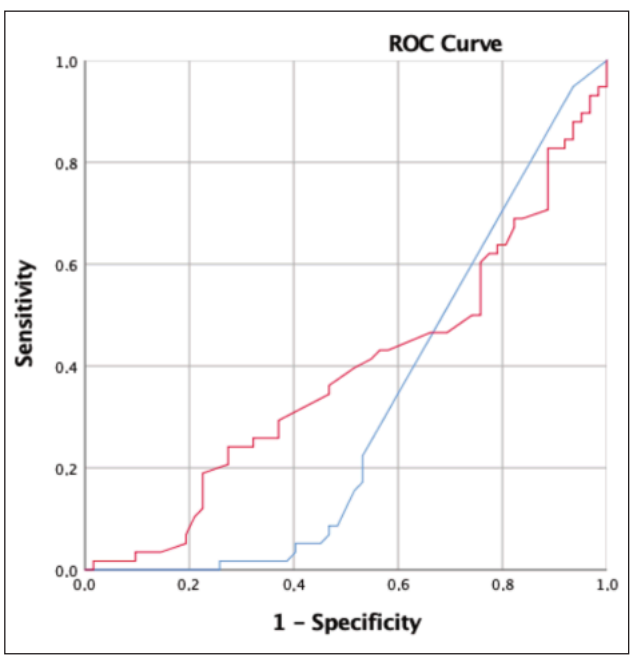

Figure 1 Receiver Operating Characteristic (ROC) curves of neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP) levels for predicting length of stay in the hospital. Blue line indicates CRP, red line indicates NLR.

Discussion

Improving length of stay prediction is an important issue in neonatal intensive care units for resource planning, reducing possibility of healthcare associated infections and improving financial efficiency. This study focused on the use of NLR as an adjunctive diagnostic tool in the prediction of length of stay in the hospital for term newborn infants with suspected neonatal sepsis. The sensitivity of the NLR was better than CRP at initial evaluation, which correlates with previous studies in other neonatal populations.[5]

In the neonatal period, the immune system develops from the fetal period. Newborns are more susceptible to infection, unlike other periods of life. This situation is even more common in preterm infants.[6] Similarly, the gestational week is the main determinant in ensuring lung maturation and reducing respiratory complications. For this reason, it should be tried to prevent births before the 39th gestational week as much as possible.[7] However, this is not always possible. As seen in our study, there is a significant difference between the increase in gestational week and the duration of hospitalisation. Similarly, the frequency of sepsis increases with the decrease in gestational weeks.[8] Infants may present with a multi systemic and sometimes even fulminant disease in which respiratory symptoms are more prominent.[9] Early detection of sepsis in these infants is vital. In our study, we found that infants with a lower gestational week had longer hospital stays in study group and more respiratory complications. The complete blood count is routinely performed for analysis in case of neonatal sepsis. Leukopenia and increased immature/total neutrophil ratio are one of the most used screening methods relating with neonatal sepsis.[10] NLR is influenced both by elevated neutrophil counts and reduced lymphocyte counts. In our study, although the median lymphocyte count was lower in the prolonged LOS group, the difference did not reach statistical significance, likely due to the wide range and variability in lymphocyte responses among neonates.

In a study evaluating the relationship between the severity of the disease and NLR in adult COVID-positive patients, it was observed that the severity of the disease was higher with high NLR. Similarly, these patients had a longer hospital stay.[11] In another adult study, the NLR of infants followed up for gram-negative sepsis were compared, and high NLR was found to be associated with both the severity of the disease and the length of hospital stay.[12] Similarly, in our study, it was observed that there was a significant correlation between high NLR and hospital stay in infants presenting with infection. However, our study differs from other studies only in that it was found to be associated with the length of hospital stay of infants.

A new study in newborns, forty-one blood culture-positive neonates and fifty-two non-septic neonates NLR values were compared and septic newborn with positive blood culture had significantly higher NLR as compared to neonates without sepsis. In the study, information was given about the length of stay of the patients in the NICU.[12] However, we think that babies with positive blood cultures have longer hospitalisation periods. In a study investigating the relationship between sepsis and haematological parameters in 166 term infants with congenital heart disease, it was found that absolute neutrophil count, systemic immune-inflammatory index and NLR increased in sepsis. The gestational weeks of the babies included in the study were similar to our study, and a relationship was found between NLR and sepsis.[13]

Like NLR, CRP is also an important inflammatory marker, and many studies have demonstrated that elevated CRP were useful biomarker for the diagnosis of neonatal sepsis.[14,15] CRP is the most elevated acute phase reactant in case of inflammation due to infection or tissue damage. CRP secretion begins 4-6 hours after inflammation and reaches its peak within 36-48 hours.[16] It is known that frequently repeated CRP values, especially in neonatal sepsis, have a predictive value of 99.7%.[17] Our study also demonstrated that number of CRP positive infants were higher in increased LOS group. At the same time, the specificity of CRP was found to be high in this study. There have been several studies that compare CRP and NLR in predicting sepsis in the newborn period.[2] Unlike other investigations, this study looked into the prediction of hospital stay duration. Neonatologists and paediatricians make decisions on newborn hospitalisation and discharge. At this point, physicians may be interested in neonatal health may pay more attention to CRP elevation or changes in CRP value. The EMA Sepsis guidelines also emphasize the CRP levels. Furthermore, white blood cell count in neonates has limited prognostic value for sepsis.[18]

We calculated the ROC curve for NLR to determine the optimal cut-off value to predict prolonged hospitalisation. Based on our analysis, an NLR cut-off value of 3.01 yielded the best balance of sensitivity and specificity. Further validation in larger cohorts will be necessary to confirm this threshold.

Because 50% of confirmed instances of sepsis were

determined to be within the normal range.[19] Maternal fever, hypertension, pre-eclampsia, mode of delivery, perinatal asphyxia, meconium aspiration, intraventricular haemorrhage, pneumothorax, haemolytic disease, reticulocytosis, seizures, and even prolonged crying can all cause changes in leukocyte and neutrophil counts, and risk factors are not always identified in the neonatal period. The neutrophil count ranges from 6,000 to 30,000/mm^3 in the first 24 hours and from 5,000 to 20,000/mm^3 after that. As a result, the presence of neutropenia is deemed more useful than neutrophilia.[20] The ratio of immature neutrophils to total neutrophils (I/T ratio) remains the most sensitive indication of newborn sepsis. The normal value is 0.16 at birth and drops to 0.12 after 60 hours; >0.2 indicates sepsis.[21] As a result, an ideal NLR value is unknown at present moment since it can change over time.

While platelets are known to play a role in the inflammatory response, neonatal platelet response to infection is complex and can be influenced by multiple perinatal factors, including maternal conditions, delivery method, and neonatal adaptation. In our study population of term infants, most had mild to moderate clinical signs, which may not have triggered a significant thrombopoietic response. Additionally, the timing of blood sampling might have occurred before a measurable change in platelet count. Another parameter whose relationship with sepsis has been investigated is mean platelet volume (MPV). Aydemir et al investigated the cut-off level for CRP, procalcitonin (PCT) and MPV in preterm sepsis.[22] ROC analysis is used to determine optimal cutoff levels for CRP, PCT and MPV. Despite they found that the diagnostic performance of CRP and PCT did not differ from each other, they were more efficacious than MPV. In another trial, day 3 MPV level is found to be a surrogate marker for the prediction of EOS and associated mortality in preterm neonates.[23] In a recent meta-analysis, the diagnostic accuracy of MPV in neonatal sepsis was investigated. MPV levels were found remarkably higher than in healthy neonates. They concluded that MPV shows a reasonable diagnostic accuracy in investigation of neonatal sepsis. In our trial we did not find any relation between MPV and increased LOS, however there may be a difference in large-scale studies.

In this trial, the LOS risk of term and near-term cases presenting with early signs of neonatal infection was analysed considering the length of hospital stay. We found that especially high NLR rate and CRP values were associated with increased LOS risk. It is important to keep in mind NLR in addition to CRP and PCT, which are classical approaches in the evaluation of these babies, as a gestational age below 39 weeks may increase the risk of LOS, especially in terms of low cost. In addition, it should be remembered that high NLR rates may be associated with prolonged hospitalisation. Paying attention to this, especially in NICUs, where the cost is high, can ensure early detection of cases and can provide both early diagnosis and lower costs.

The most significant disadvantage of this study is that it was designed retrospectively and included a small number of patients. To improve the performance of the NLR in predicting length of stay we have to organise prospective, multicenter studies with large case series and the recording of NLR values by day are required. We believe that by analysing NLR values changing by day, we will be able to gain a better understanding of the duration of hospitalisation.

Conclusion

CRP remains a highly specific and robust marker for assessing the severity and progression of early neonatal infection. However, the NLR offers the advantage of being immediately available from routine CBC without additional cost or laboratory delay. NLR may be particularly useful in the early phase of infection, before CRP elevation is detectable. Therefore, we propose that NLR should be considered as a supportive biomarker alongside CRP, especially in early evaluation. This study is the first cohort study that evaluates the value of NLR and CRP for predicting of length of stay in term neonates with neonatal infection. Our results emphasize that, both NLR and CRP are effective in predicting length of stay in this group. However, the results of this study need to be confirmed by further clinical trials in a large number of newborn infants.

Conflict of Interest

All authors declare no conflict of interest.

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