ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Evaluation of Red Blood Cell Distribution Width-Platelet Ratio as an Early Predictor of Late-Onset Sepsis in Preterm Infants

Preterm İnfantlarda Geç Sepsis Erken Tanısında Eritrosit Dağılım Hacmi/Trombosit Oranının Değerlendirilmesi

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Abstract

Introduction: Late-onset sepsis (LOS) is a major cause of death and neurodevelopmental impairment in preterm infants. In this study, our aim was to review the role of red cell distribution width to platelet ratio (RPR) to predict LOS in preterm infants.

Materials and Methods: Preterm infants with ≤366/7 gestational weeks who were admitted to the neonatal intensive care (NICU) between January 2018 and 2020 were accepted in this observational cohort study. LOS group had culture-proven LOS and the control group with no LOS during their NICU stay. Complete blood cell parameters were recorded on the day of culture growth in the groups. The study cohort was classified into two groups according to the type of the growing microorganism and the RPR levels were then evaluated in intra- and inter-group analyses.

Results: Eighty -five infants were included in the final analysis. RPR values were significantly higher in the LOS group ($p<0.001$) In the subgoup analyses, RDW and RPR values were significantly higher in the group with Gram-negative sepsis ($p<0.001$). The sensitivity and specificity of an RPR cutoff value of 0.17% were found to be 60% and 92% ($p<0.001$), respectively for predicting LOS.

Conclusion: This study defined that RPR is a practical and useful marker to predict LOS in preterm infants. Future prospective studies with large study groups are needed to evaluate the role of RPR in the prediction of late-onset sepsis.

Öz

Giriş: Preterm infantlarda geç sepsis nörogelişimsel gerilik ve ölümün önemli nedenlerinden biridir. Bu çalışmadaki amacımız, eritrosit dağılım genişliği/trombosit (RPO) oranının geç sepsis erken tanısındaki rolünü araştırmaktır.

Gereç ve Yöntem: Gözlemsel kohort çalışmaya Ocak 2018 ve 2020 yılları arasında yenidoğan yoğun bakım ünitesinde yatarak tedavi görmüş ≤366/7 gestasyonel haftanın altındaki preterm infantlar dahil edilmiştir. Geç sepsis grubuna kültür kanıtlı sepsis izlenenler dahil edilirken kontrol grubuna geç sepsis atağı izlenmemiş hastalar dahil edilmiştir. Kültür üremesi ile eş zamanlı alınmış olan tam kan parametreleri kaydedilmiştir. Ayrıca çalışma grubu kültürde üreme izlenen mikroorganizma tipine göre gruplandırılmış ve gruplar arası ve grup içi analizleri kaydedilmiştir.

Keywords
Late-onset sepsis, preterm infants, red cell distribution width to platelet ratio

Anahtar kelimeler
Geç sepsis, preterm infant, eritrosit dağılım genişliği trombosit oranı

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Bulgular: Son analize seksen beş infant dahil edilmiştir. Geç sepsis grubunda RPO oranları istatistiksel anlamlı olarak yüksek izlendi (p<0,001). Subgrup analizinde RDW ve RPO değerleri Gram-negatif sepsis grubunda belirgin yüksek izlendi (p<0,001). Geç sepsis öngörmede RPO eşik değeri olarak %0,17 degerinin sensitivite ve spesifitesi sırasıyla %60 ve %92 olarak izlendi.
Sonuç: Bu çalışmada geç sepsisi öngörmede RPO değerinin pratik ve yararlı olduğu gösterildi. RPO değerinin geç sepsisi öngörmedeki rolü ile ilgili prospektif ve geniş çalışma grupları ile yapılacak çalışmalara ihtiyaç vardır.

Introduction

Late-onset sepsis (LOS) is most frequently determined as neonatal sepsis begining after 3 days of postnatal age, represents a crucial cause of morbidity and mortality in preterm infants (1-4). The frequency of LOS is inversely associated with birth weight (BW) and gestational age. In parallel with the development in the survival rates of preterm infants, there is an increase in other well-known risk factors for LOS such as long mechanical ventilation and intravascular catheterization days, extended duration of parenteral nutrition, and hospitalization days (1,2,5). The incidence of culture-proven LOS alters amongst neonatal units from 21-36% (6,7). The main pathogens causing LOS are coagulase-negative *Staphylococci* (CONS), followed by Gram-negative bacteria, and fungi. In the way of toxin production, CONS are not as serious as Gram-negative bacteria and therefore short-term infectious complications and mortality are monitored rarely with CONS. However, studies implicate the risk of neurodevelopment sequelae was independent of the type of pathogen (1,2,6).

Rapid diagnosis of LOS is still a problematic condition for clinicians because the featuring signs are often non-specific (8). Blood culture is the gold standard for diagnosing sepsis; however, this 'gold standard' testing method has some disadvantages. These disadvantages involve; it may produce false-positive results in addition to false-negative results and also it takes time to get the results (2,3). In neonatal sepsis, various molecules have been studied as possibly useful prognostic markers like procalcitonin, IL-6, IL-8, CD64, presepsin, raised ratios of anti- and pro-inflammatory cytokines (e.g. IL-10/TNF-α and IL-6/IL-10). Also currently molecular-based methods have risen as diagnostic methods for neonatal sepsis (2,4,9). Red blood cell distribution width (RDW) is a quantitative measure of variability in the size of circulating erythrocytes. The crucial role of RDW has been reported in a range of inflammatory diseases in adults and neonates (1,3,10-13). In inflammation, proinflammatory cytokines and high oxidative stress alter both erythropoiesis and erythrocyte maturation and by entering newer reticulocytes in circulation, RDW is increasing. In addition, inflammation tissue factor causes reduced platelet count (1,3). In recent years many reports have shown that red cell distribution width to platelet ratio (RPR) is a practical and helpful marker of inflammatory diseases (3,10,14). In the present study, the role of RDW and RPR parameters, which are parts of a complete blood count analysis, were interrogated to predict LOS in preterm infants.

Materials and Methods

An observational cohort study was attended at the University of Health Sciences Turkey, Bursa Yüksek İhtisas Training and Research Hospital between January 2018 and 2020 upon approval of the study by the Local Ethics Committee (approval number: 05-18, date: 05.05.2021). The study cohort was divided to two groups: LOS group was preterm infants who were at $\leq 36^{6/7}$ gestational weeks and had culture-proven LOS during their neonatal intensive care unit (NICU) stay and the control group were preterm infants who were at $\leq 36^{6/7}$ gestational weeks with no LOS attack during NICU stay. The definition of neonatal sepsis was the presence of clinical signs of sepsis with positive blood culture, and LOS was defined as a sepsis attack, which developed between day 4 and 30 of postnatal life and infants with more than one episode of LOS only the first LOS episode was taken into account. The exclusion criteria were as follows: infants with previous blood transfusion before the first LOS episode; maternal moderate/severe anemia; maternal medications that may alter the fetal hemopoietic system; positive family history of hematologic diseases such as thalassemias; infants with major congenital anomalies, hydrops fetalis, intrauterine transfusion, perinatal asphyxia, grade 3 and 4 hemorrhage and refusal of parental consent.

Details of maternal medical history, prenatal demographics, antenatal steroid administration

gestational age, birth weight, gender, mode of delivery, Apgar score at 1 and 5 minutes, the type of microorganism grown in blood culture, were recorded. Blood cultures were examined using the fully automatic BACTEC method by BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany).

Two milliliters of blood were collected from a peripheral vein into a K3 EDTA tube (tripotassium ethylenediaminetetraacetic acid) and counts were performed within 1 hour of sample collection. Hematological parameters were determined with a Sysmex-XT-2000i counter (Sysmex, Kobe, Japan). Complete blood cell parameters, including the RDW, platelet count, RPR, and blood cultures were recorded on the day of culture growth in the study group. Complete blood cell parameters taken routinely on the postnatal 10th day were also recorded in the control group. The RPR values recorded in the blood samples received on the day of culture growth were compared between the groups.

Statistical Analysis

Statistical analysis was accomplished using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA). The results are shown as median (interquartile range) for the variables showing non-Gaussian distribution and mean ± standard deviation (SD) for data showing normal distribution. The Student's t-test was applied for group comparisons of normal distributions, and Mann-Whitney U-test was applied for group comparisons of non-normal distributions. The chi-square test and Fisher's exact test were consumed for the comparison of categorical variables. The Wilcoxon-signed test was applied to correlate paired data. Logistic regression analysis was achieved to consider the association between RDW and mortality. The analysis involved factors that were established in the literature to affect mortality, including gestational week and birth weight were included in the analysis. The predictive power of different variables was defined by the receiver operating characteristic (ROC) curve. A p-value of <0.05 was studied statistically significant.

Results

Of the 20710 live births, a total of 1205 preterm infants were accepted to the NICU during the study period. After excluding infants with exclusion criteria, 85 infants were included in the final analysis. Forty-five (53%) of these infants had LOS, while 40 (47%) infants had no LOS attack during their NICU stay. A detailed flowchart of the study population is shown in Figure 1.

The mortality rate was significantly higher in group 2 (p=0.001). Demographic characteristics of the study population are shown in Table 1.

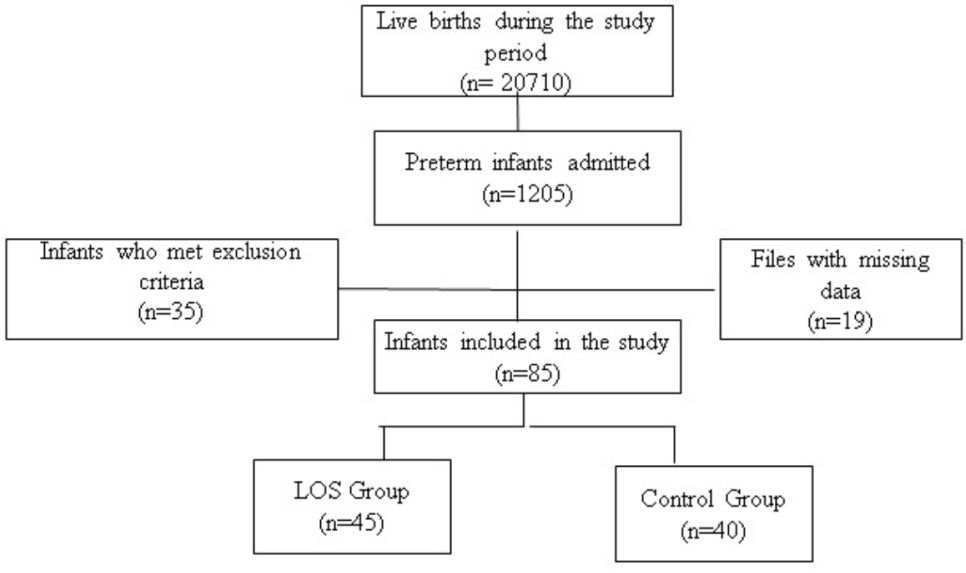

Figure 1. Flowchart of the study population.

Table 1. Neonatal and maternal characteristics of the study population			
	LOS group (n=45)	Control group (n=40)	p
GA at birth, wk [median (IQR)]	28 (27-31)	30 (28-32)	0.08[a]
BW, g [median (IQR)]	1130 (865-1415)	1367 (1010-1892)	0.06[a]
Sex, n (%)			
Male	24 (53)	24 (60)	0.5[b]
Female	21 (47)	16 (40)	
C/S delivery, n (%)	39 (87)	32 (80)	0.4[b]
Apgar score, median (IQR)			
at minute 1	7 (6-8)	6 (6-7)	0.6[a]
at minute 5	8 (7-9)	8 (7-9)	0.6[a]
Antenatal steroid, n (%)			
Single/repeat course	18 (38)	23 (59)	0.8[b]

[a] Mann-Whitney U test, [b]Chi-square test, GA: Gestational age, BW: Birth weight, C/S: Cesarean section, wk: Week, LOS: Late onset sepsis

When the whole blood cell parameters and CRP levels were taken at the same time with the blood sample in which the culture growth was monitored, the platelet levels were significantly lower in the LOS group (p<0.001). CRP and RDW values were significantly higher in the LOS group (p<0.001 for both). When RPR values were compared in both groups, RPR values were significantly higher in the LOS group (p<0.001). When considered in terms of other hematological parameters, no significant difference was observed between the two groups.

Laboratory findings of the study groups are given in Table 2. Cases with LOS were divided into subgroups according to the type of microorganism grown in culture, as those with Gram-positive and negative growth. Gram-positive growth was observed in 64% of cases with LOS, and Gram-negative growth was observed in 36%. Cases with LOS were divided into subgroups according to the type of microorganism grown in culture, as those with Gram-positive and negative growth. In the subgroup analysis, hemoglobin and platelet values were significantly lower in the group with Gram-negative growth in culture (p=0.003, p<0.001, respectively). CRP value was significantly higher in the group with Gram-negative growth (p=0.03). In addition, RDW and RPR values were significantly higher in the group with Gram-negative growth (p<0.001). Laboratory findings of the subgroups are given in Table 3.

Logistic regression analysis defined a positive association of RPR with LOS when adjusted for the gestational week and birth weight (adjusted odds ratio: 573.7; 95% confidence interval: 9.4-34778; p=0.002). Reciever-operating characteristic (ROC) analysis was accomplished to determine the optimal threshold values of RPR for predicting LOS, the area under the curve (AUC) was determined to be 0.762. The sensitivity and specificity of an RPR value of 0.17% were found to be 60% and 92% (p<0.001), respectively (Figure 2).

Discussion

Premature infants are at high risk for LOS because of many factors as well as immaturity of their innate immune system, extended hospitalization days with frequent invasive procedures. LOS is a major cause of death and neurodevelopmental impairment in premature infants (15). In recent years many researchers reported biomarkers that are potentially

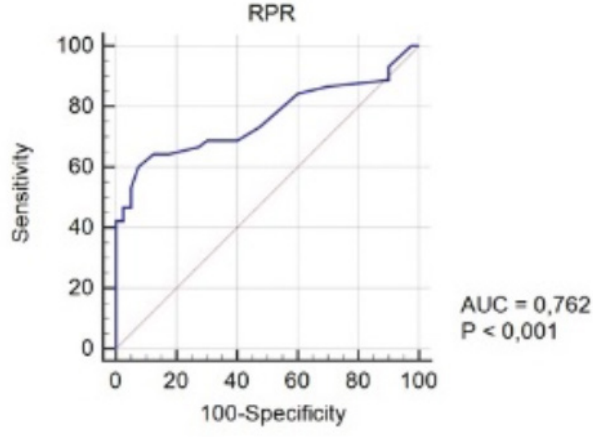

Figure 2. Receiver operating characteristic curves for prediction late-onset sepsis by RPR.

valuable both for diagnosis of LOS and prediction of its outcome. Unfortunately, many of these biomarkers are not readily accessible in many neonatal intensive care units (9,16,17). In the present study, there was a significant increase in RPR during the LOS episode. An RPR value of 0.17% was determined to be a reliable cut-off to predict LOS. To the best of our knowledge, this is the first study that defined RPR as an early predictor of LOS. Our findings demonstrate that RPR, which can be assessed practically in the complete blood count as part of the routine sepsis evaluation, may play a role in the diagnosis of LOS.

The most common pathogens in LOS are coagulase-negative staphylococci (CONS), S. aureus, Gram-negative bacteria (*Klebsiella* spp., *Pseudomonas* spp., *E. Coli*, and *Enterobacter* spp., respectively), and *Candida* spp. (18). Although CONS takes the first place as causative organisms in LOS, Gram-negative infections are associated with a higher risk of morbidity and mortality (19). In most cases, the clinic signs of LOS are often non-specific but can immediately progress to septic shock and death within hours of onset. Despite paramount importance to find a tool for prediction LOS, it is yet to be found (2,9). In recent years, many researchers have reported many biomarkers and CRP for the early diagnosis of sepsis. Cytokines, such as IL-6, IL-1b, SIL2R, IL-8, and TNF-α, cell surface antigens as biomarkers, such as CD11b, CD64, sCD163 were reported as early predictors of sepsis (18,20,21). A recently published study showed that IL-6 and PCT give important information about the severity of disease and mortality risk in LOS (22). Mostly in NICUs, these biomarkers are not readily available because they are expensive, and not practically accessible (1,9). Because of this, simple and easily available predictive markers are needed. With this point of view, RPR is practically accessible marker, which is a part of the routine complete blood-count parameters. In this study, it is found that an RPR value of 0.17% was found to be a reliable cut-off to predict LOS.

Thrombocytopenia is a frequent pathology in NICUs and is seen in 18–35% of neonates admitted to NICUs. Neonatal thrombocytopenia has been defined into two groups according to the time of onset: early-onset (EOT), which is within 72 h of life, and late-

Table 2. Laboratory findings of the study and control groups

	LOS group (n=45)	Control group (n=40)	p
WBC count ($10^3/\mu$L) [median (IQR)]	11 (6.1-16.7)	10 (7.2-14.7)	0.9[a]
Hemoglobin (g/dL) (mean ± SD)	12.5±2.3	14.6±3.6	**0.001**[b]
Platelet count ($10^3/\mu$L) [median (IQR)]	68 (27-224)	228 (123-279)	**<0.001**[a]
MPV (fL) (mean ± SD)	9.5±1.4	9.6±1.2	0.6[b]
CRP (mg/L) [median (IQR)]	78 (47-105)	5 (3.5-6)	**<0.001**[a]
RDW (%) [median (IQR)]	19.3 (17.5-21.2)	16.4 (15.9- 19.4)	**<0.001**[a]
RPR (%) [median (IQR)]	0.27 (0.07-0.73)	0.08 (0.05-0.13)	**<0.001**[a]

[a]Mann-Whitney U test, [b]Student's t test, WBC: White blood cell, IQR: Interquartile range, MPV: Mean platelet volume, CRP: C-reactive protein, RDW: Red cell distribution width, RPR: Red cell distribution width to platelet ratio, LOS: Late onset sepsis, SD: Standard deviation

Table 3. Laboratory findings of the subgroups

	Gram positive group (n=29)	Gram negative group (n=16)	p
WBC count ($10^3/\mu$L) [median (IQR)]	12.6 (5.6-21.1)	8.2 (6.2-12.7)	0.2[a]
Hemoglobin (g/dL) (mean ± SD)	13.1±2.6	11.3±1.3	**0.003**[b]
Platelet count ($10^3/\mu$L) [median (IQR)]	127 (82-257)	17 (8-29)	**<0.001**[a]
CRP (mg/L) (mean ± SD)	68.4±41.3	108.3±62.6	**0.03**[b]
RDW (%) (mean ± SD)	18.2±2.06	21.5±1.4	**<0.001**[b]
RPR (%) [median (IQR)]	0.12 (0.06-0.23)	1.3 (0.62-2.75)	**<0.001**[a]

[a] Mann-Whitney U test, [b]Student's t-test, WBC: White blood cell, IQR: Interquartile range, MPV: Mean platelet volume, CRP: C-reactive protein, RDW: Red cell distribution width, RPR: Red cell distribution width to platelet ratio, SD: Standard deviation

onset (LOT), after 72 h of life (23). The most frequent causes of thrombocytopenia are prematurity, sepsis, NEC and asphyxia. In a study, it was demonstrated that the mean platelet count in Gram-negative sepsis was significantly lower than gram-positive infection (23). In another recent study sepsis by Gram-negative bacteria was defined as an independent predictor for severe thrombocytopenia (24). RPR is a ratio (red cell distribution width to platelet ratio) therefore when the decrease in platelet count is evident, the RPR rate increases. In this study, the RDW value was higher in the group with Gram-negative growth than the group with Gram-positive growth, while platelet values were also significantly lower. Therefore, the RPR rate was significantly higher in infants with Gram-negative growth. In cases with a high RPR value, Gram-negative sepsis may be the cause of LOS and the empirical treatment plan can be adjusted accordingly.

Study Limitations

There are several limitations of the present study. Firstly, the study is a retrospective study accordingly it is difficult to explain presumed conclusions. Secondly, because of the relatively small sample size, results can be misleading. And at last, in addition to CRP, procalcitonin which is an important and available biomarker in many NICUs, could have been compared with RPR. Concerning the strengths of this study, to our knowledge, this study is the first study that evaluates the association of RPR and LOS, and the results of this study can shed light on future prospective studies.

Conclusion

This study defined that RPR is a practical and useful predictor to predict LOS in preterm infants. In addition, higher RPR values were observed in the group with Gram-negative growth compared to those with gram-positive growth. These results will be essential in the early and adequate management of LOS in preterm infants. Future prospective studies with large study groups are needed to analyze the role of RPR in the prediction of late-onset sepsis.

Ethics

Ethics Committee Approval: An observational cohort study was attended at the University of Health Sciences Turkey, Bursa Yüksek İhtisas Training and Research Hospital between January 2018 and 2020 upon approval of the study by the Local Ethics Committee (approval number: 05-18, date: 05.05.2021).

Conflict of Interest: No conflict of interest was declared by the authors.

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