Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.01.023 2020;27(1):23-8

Original Article

Can neutrophil-lymphocyte ratio be a predictor of late-onset sepsis in preterm infants?

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Abstract

Aim: Late-onset sepsis (LOS) persists to be a crucial reason of morbidity and mortality in preterm infants. Studying the effect of neutrophil-lymphocyte ratio (NLR) value in predicting the LOS episode and relation with the type of the growing microorganism were the aim of this study.

Material and Methods: Preterm infants were classified as two groups in accordance with having culture proven LOS during the stay in neonatal intensive care unit. Premature infants with culture proven LOS was defined as study group and infants with no-LOS as control group. Study group is consisting of two subgroups in terms of the growing microorganism type. NLR values were compared within and between the groups.

Results: Overall, final analysis were applied to 116 infants. NLR values were raised in the study group during LOS episode ($p<0.001$). NLR value of the Gram-positive sepsis group (Subgroup 1) was significantly higher in comparison to Gram-negative sepsis group (Subgroup 2) (median: 2.4 and 1.8, respectively, $p = 0.02$). ROC analysis showed that area under the curve (AUC) of NLR was 0.723 ($p<0.001$) and cut-off point optimized for sensitivity of 68% and a specificity of 82% was stated as NLR ≥1.57 for conforming LOS.

Conclusions: These findings demonstrated that in preterm infants, NLR values rise for the duration of a LOS episode. We believe that NLR value may be a helpful for the prediction of LOS in preterm infants.

Keywords: Late-onset sepsis; neutrophil/lymphocyte ratio; preterm

INTRODUCTION

Neonatal sepsis is characterized by symptoms of infection mostly with bacteremia in the first month of life (1). Because of the lack of specific findings of sepsis in the neonatal period, sepsis diagnosis is a challenging decision for clinicians (2). Despite all technological advances and development in neonatal medicine, late-onset sepsis (LOS) already an important cause of morbidity and mortality in neonatal intensive care units (NICU) (3). The frequency of LOS in very low birth weight infants is approximately 15-25% in developing countries. This is especially evident in premature infants because of their prolonged hospitalization, and their need for invasive procedures during their NICU stay makes them vulnerable for hospital-acquired infections (4,5).

The gold standard of the sepsis diagnosis is the identification of the responsible microorganism in blood cultures; therefore, this is a time-consuming method and at least 2-3 days of culture is necessary to identify the microorganism in the blood. Thus, diagnostic biomarkers become seriously important for the early determination of LOS in preterm infants (6). Clinical findings of sepsis are frequently non-specific and microbiological culture findings are not accessible in the first 48–72 hours of infection (1,6). Currently many biomarkers such as C-reactive protein (CRP), procalcitonin, IL-6, IL-8, and CD64 are used as early diagnostic markers of sepsis in neonates a simple bedside marker can be applied to evaluate the severity of sepsis (7,8). Recently, there has been an increasing trend by using complete blood count (CBC) and ratio of parameters as markers of inflammation in many different diseases (9,10). In the present study we aimed to assess NLR ratio during LOS attack and the

Received: 14.01.2020 **Accepted:** 02.02.2020 **Available online:** 17.02.2020
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role of NLR ratio in predicting the group of responsible pathogens of LOS in preterm infants.

MATERIAL and METHODS

The presented study designed as a retrospective-cohort study and was initiated at the University of Health Sciences, Bursa Yuksek Ihtisas Teaching Hospital between January 2016 and January 2018 upon approval of the study by the local ethics committee. The preterm infants (<37 gestational weeks) who hospitalized to NICU, were admitted to the study. Premature infants with culture proven LOS during their hospitalization defined as study group and infants with no-LOS during their hospitalization defined as the control group. According to the type of bacterial growth in the blood culture, two subgroups were constituted from the study group. In subgroup 1 preterm infants with Gram-positive bacterial growth and in subgroup 2 preterm infants with Gram-negative bacterial growth were included. LOS was determined as a sepsis episode with the presence of clinical signs with a positive blood culture occurred between 4 and 30 days of life (2). For consideration of Staphylococcus epidermidis as a pathogen rather than a contaminant, it was isolated in two separate sets of blood cultures from two different sites. The exclusion criteria were as follows: central nervous system malformations, congenital heart disease, metabolic disorders, chromosomal abnormalities and birth asphyxia.

Maternal and fetal demographic data's as mode of delivery, antenatal steroid administration, gestational age, birth weight, sex, Apgar scores at 1 and 5 minutes were recorded. Neonates with culture proven LOS, type of microorganisms and mortality data were documented. For the analyzing of blood cultures, BACTEC method by BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany) were used.

Blood samples of the study group were obtained from at the time of the sepsis episode when the blood culture growth was observed. Complete blood count parameters belong to control group at fifteenth day after birth which also correspond average sepsis beginning day of the LOS group. Within 30 minutes of the sample collection, at least two milliliters of blood were obtained from a peripheral vein into a K3 EDTA tube (tripotassium ethylenediaminetetraacetic acid tube) and counts were completed. Hematological parameters were measured by an automated analyzer (Sysmex-XT-2000i, Kobe, Japan). Documented hematologic parameters were analyzed within and between the groups.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 22.0 software program (SPSS Inc., NY, USA). The Shapiro-Wilk test was used to evaluate the type of distribution of quantitative variables. Normal distributed results were defined as mean ± standard deviation, abnormal distributed results were defined as median (and interquartile range (IQR) values. The categorical values were expressed as number and percentage. Chi-Square analysis and Fisher's exact test were applied to compare categorical variables. Parametric/ non parametric tests were applied to compare quantative variables (Student t test / Mann-Whitney U test). The predictive power of different variables was assessed by receiver operating characteristic (ROC) analysis. Positive and negative likelihood ratios were evaluated to assess the clinical usefulness of NLR. Statistical significance was set at $p< 0.05$.

RESULTS

Overall 142 premature infants were included in the study. Twenty-six premature infants excluded according to the exclusion criteria (Nineteen patients with missing data, 2 patients with congenital heart disease and 2 patients with birth asphyxia, 3 patients with metabolic disorder, chromosomal abnormality and central nervous system malformations) the files of 116 infants were analyzed. Seventy-six infants were included in the study group and 40 infants were included in the control group. In the subgroup analyses of the study group; 42 (55%) of these infants were in Gram-positive sepsis subgroup and 34 (44%) infants were in Gram-negative sepsis subgroup. Flowchart of the study population and the demographic characteristics of the study (LOS group) and control groups are presented (Figure1 and Table 1). According to the demographic characteristics between the subgroups no statistically significant differences were found.

In subgroup 1, 38 (%90) of them Coagulase-negative staphylococci, 4 (%10) of them Staph. aerius, were the isolated pathogens. In subgroup 2 of 15 (%44) Klebsiella pneumonia, 7 (%21) Escheria coli, and 2 (%6) Serratia marcescens, 4 (%12) Acinetobacter baumani, 6 (%18) Pseudomonas aeruginosa were the isolated pathogens.

After evaluation of complete blood count parameters and CRP levels, it was found that the NLR was significantly higher in study group compared with control group (mean 3.2±2.5 vs 1.4±0.2, p<.001). Laboratory findings of the study (LOS group) and control groups are demonstrated in Table 2. NLR was found significantly higher in subgroup 1 when compared with subgroup 2 (median: 2.4, IQR:1.8-5.8; median: 1.8, IQR: 1.4-4.8, respectively, p=0.02). In addition, the CRP was found to be significantly higher and platelet levels were found to be significantly lower in subgroup 2 (p=0.002, p=0.01 respectively). The comparison between the two subgroups in terms of other hematological parameters showed no significant difference (Table 3).

For confirming sepsis and Gram-positive sepsis to determine optimal cut off point of NLR, ROC analysis was performed and in Figure 2 and Figure 3 results of the ROC analyses were shown. After the ROC analyses the area under the curve (AUC), p-values, cutoff point optimized for sensitivity and specificity, 95% CI determined for the NLR value. ROC analysis indicated that AUC of NLR was 0.723 (p<0.001) cutoff point optimized for sensitivity and

Table 1. Neonatal and maternal characteristics of the study groups

	Late-onset Sepsis Group (n=76)	Control Group (n=40)	P
GA at birth, wk mean ± SD	29 ± 3	28 ± 3	0.6[a]
BW, g median (IQR)	1127 (840-1434)	1230 (990-1475)	0.2[b]
Sex, n (%)			
Male	40 (53)	22 (55)	0.9[c]
Female	36 (47)	18 (45)	
C/S delivery, n (%)	57 (75)	27 (68)	0.4[c]
Apgar score, median (IQR)			
Minute 1	6 (5-7)	6 (6-7)	0.8[a]
Minute 5	8 (7-8)	8 (7-8)	0.8[a]
Antenatal steroid, n (%)	42 (55)	23 (57)	0.8[c]
Preeclampsia, n (%)	32 (42)	14 (34)	0.2[c]
IUGR, n (%)	16 (21)	6 (14)	0.1[c]
Mortality, n (%)	16 (21)	4 (10)	0.1[b]

a Student-T test, b Mann-Whitney U test, c Chi Square test
GA: gestational age; wk: week; SD: standard deviation; IQR: interquartile range; BW: birth weight; C/S: cesarean section; IUGR: intrauterine growth restriction

Table 2. Laboratory findings of the study groups

	Late-onset Sepsis Group (n=76)	Control Group (n=40)	P
WBC count (10^3 /µL) mean ± SD	14.2 ± 11	13.8 ± 3.3	0.7[a]
Neutrophil count (10^3 /µL) mean ± SD	10.5 ± 8.9	7 ± 1.9	0.1[a]
Lymphocyte count (10^3 /µL) mean ± SD	3.2 ± 2.8	5 ± 1.3	<0.05[a]
NLR mean ± SD	3.2 ± 2.5	1.4 ± 0.2	<0.001[a]
Platelet count (10^3 /µL) mean ± SD	167 ± 140	382 ± 72	<0.001[a]
MPV (fL) mean ± SD	9.2 ± 1.1	8.9 ± 1.1	0.06[a]
CRP (mg/L) median (IQR)	53 (18-96)	3.7 (3-4.9)	<0.001[b]

[a] Student-T test, b Mann-Whitney U test, c Chi Square test
GA: gestational age; wk: week; SD: standard deviation; IQR: interquartile range; BW: birth weight; C/S: cesarean section; IUGR: intrauterine growth restriction

Table 3. Laboratory findings of the subroups during the sepsis episode.

	Gram-positive Group (n=42)	Gram-negative Group (n=34)	P
WBC count (10^3 /µL) mean ± SD	13.5 (7.5-20)	10.1 (5.6-16.8)	0.2[a]
Neutrophil count (10^3 /µL) mean ± SD	7.9 (3.4-12)	3.9 (1.8-10)	0.1[a]
Lymphocyte count (10^3 /µL) mean ± SD	2.2 (1.1-4.5)	2.6 (1.4-4.8)	0.4[a]
NLR mean ± SD	2.4 (1.8-5.8)	1.8 (1.4-4.8)	0.02[a]
Platelet count (10^3 /µL) mean ± SD	193 (99-301)	57 (18-201)	0.01[a]
MPV (fL) mean ± SD	9.4 ± 1.1	9.7 ± 1.1	0.2[b]
CRP (mg/L) median (IQR)	48 ± 38	84 ± 57	0.002[b]

[a]Mann-Whitney U test, bStudent-t test.
WBC: white blood cell; IQR: interquartile range; SD: standart deviation; NLR: Neutrophil- lymphocyte ratio; MPV: mean platelet volume; CRP: c-reactive protein

specificity was determined as NLR ≥1.57 for confirming LOS. Sensitivity level was 68 (95%CI: 55.4-77.5), specificity level was 82 (95%CI: 67.2-92.7). ROC analysis was applied to determine the optimal cut-off value of NLR for confirming Gram positive sepsis, the AUC was found to be 0.64. The sensitivity and specificity of NLR value of 1.48 were found to be 78 (95%CI: 64.2-89.7) and 52 (95%CI: 37.9-72) (p=0.019), respectively.

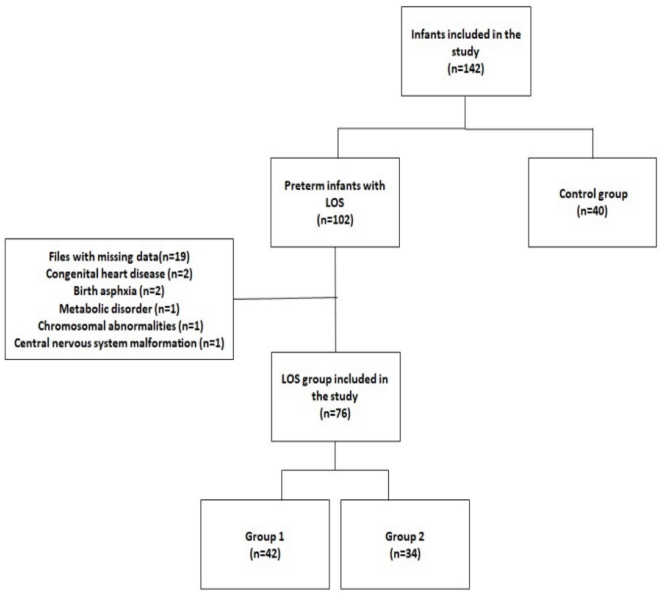

Figure 1. Flow chart of the study population

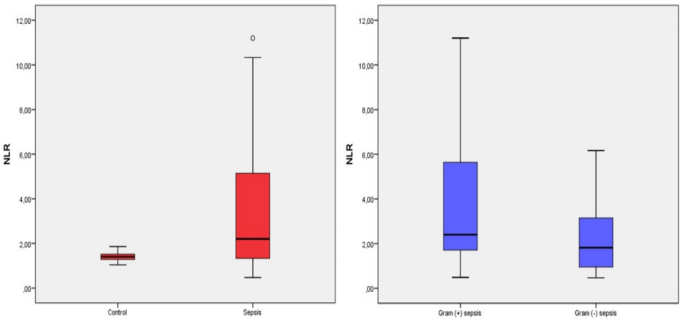

Figure 2. Distribution of Neutrophil- Lymphocyte ratio (NLR) in study groups with box-plot diagram

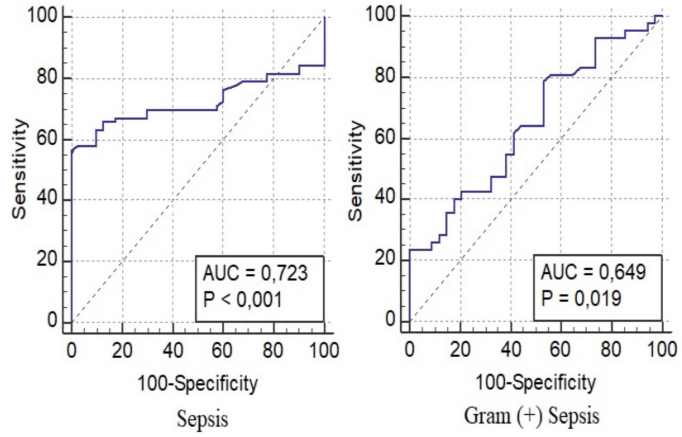

Figure 3. Reciever operating characteristic curves (ROC) for prediction of sepsis and late-onset Gram-positive sepsis by neutrophil- lymphocyte ratio (NLR)

DISCUSSION

Despite the progress in survival rates of preterm infants due to improved perinatal care in recent years, LOS is still a life-threatening morbidity of survival and long-term outcomes (11). In accordance with the recent data, frequency of neonatal sepsis in NICU is 9.3% -25% in our country (2). Therefore, early and accurate detection of sepsis becomes crucial. Even though blood culture is accepted as the gold standard in the diagnosis of sepsis, isolation of agent from the culture cannot be possible in most of the neonatal sepsis cases. The frequency of culture proven sepsis is with 35 % probability in most centers (12). The blood culture results cannot be obtained within 48-72 hours of the infection and the timing of the treatment is crucial for the prognosis of sepsis in neonates. Due to difficulties in the diagnosis of neonatal sepsis, different biomarkers have been investigated for the early and reliable diagnosis (13, 14). In this study it was shown that a simple test such as a parameter of complete blood count can be used as a practical test to diagnose LOS. An NLR value of ≥1.57 was determined as a cut-off value to confirm the diagnosis. In particular, it was demonstrated that a significant rise in NLR value with the diagnosis of Gram-positive LOS group which is the most common LOS agent in NICUs (2, 4). In this study it was defined especially for the confirmation of the Gram-positive infections, NLR value as an early practical test to predict LOS.

The NLR is a complete blood count parameter and defined practically, which is calculated from the ratio of neutrophil count to lymphocyte count. The increase in NLR may be due to increased neutrophil count or decreased lymphocyte count. Association of this increase which indicates inflammation was used as a prognostic marker for the follow up sepsis in adult patients (15, 16). In a similar study, in cases with NLR increase in suspected bacterial sepsis, blood culture growth was detected and diagnostic accuracy was demonstrated (17). Increased NLR as an indicator of inflammation has been used in the diagnosis of inflammatory diseases like infective endocarditis and acute pancreatitis in adult patients (18, 19).In a recent study with a pediatric cohort, an increase in NLR and MPV was shown in CNS tumors and correlation with presence of tumors in children under 3 years old was detected (20). There are limited studies defining NLR as a prognostic marker in the neonatal period. Patent ductus arteriosus (PDA) is considered as sterile inflammation and is associated with low lymphocyte count which is defined as a signal of physiological stress. In a recent study with the hemodynamically significant PDA patients, increasing NLR value was defined (21). In neonatal sepsis studies, increasing NLR values at the time of sepsis were demonstrated and NLR cut-off value for the diagnostic test of early-onset sepsis (EOS) is found to be significantly high (NLR ≥6.76) (8). However, in our study, NLR cut-off value of LOS group (NLR ≥ 1.57) was lower when compared with the EOS studies. This difference may be the effect of the higher neutrophil counts in the early days of life and may cause to be relative higher NLR values in neonates. Another study investigating the NLR cut-off value for conforming neonatal sepsis found a higher value (NLR ≥ 2.7) and this may be a result of the heterogeneity of the study group which consists of both EOS and LOS groups (7). Recently in another study investigating NLR as a predictor of culture proven LOS, the cut-off values (NLR ≥1.77) were similar with the presented study, but the increase in NLR was more significant in the Gram-negative sepsis group (22). Difference of the presented study from other studies is that increase in NLR value was not significant as Gram-positive sepsis than in Gram-negative sepsis group. This result may be caused by the low neutrophil count thereby less increase in NLR value in Gram-negative LOS in the neonatal period. As shown in a study conducted in very low birth weight (VLBW) infants, neutropenia is mostly prevalent in Gram-negative LOS when compared with Gram-positive LOS (23). In the presented study it was demonstrated that NLR value can be used as a simple and easy test in the diagnosis of LOS in preterm infants.

In the presented study there are some limitations. Firstly, although we used an extremely reliable neonatal database that determined all the premature infants with LOS but it is a retrospectively designed study. Second limitation of the study is that our study group is relatively small therefore it cannot reflect the general population. With regard to the strengths of our study, many factors that may have affected NLR were excluded, and the association between LOS and NLR could be evaluated more accurately. Other point of the strength of the study, only culture proven LOS cohort was evaluated and therefore the diagnosis of the LOS was definitive.

CONCLUSION

In conclusion, in the present study, we demonstrated that NLR value is a practical test that can be used to exclude LOS in premature infants. Moreover we defined a reliable NLR value for confirming Gram positive sepsis which is more frequent in NICUs. We think, these findings will be valuable in early and efficient management of LOS in premature infants. Further prospectively randomized controlled studies in large populations are warranted to better understand utility of this potential..

Competing interests: The authors declare that they have no conflict of interest.

Financial Disclosure: There is not any sources of financial assistance.

Ethical approval: The presented study designed as a retrospective-cohort study and was initiated at the University of Health Sciences, Bursa Yuksek Ihtisas Teaching Hospital between January 2016 and January 2018 upon approval of the study by the local ethics committee.

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REFERENCES

1. Bekhof J, Reitsma JB, Kok JH et al. Clinical signs to identify late-onset sepsis in preterm infants. Eur J Pediatr. 2013;172:501-8.

2. Satar M, Arisoy AE, Celik IH. Turkish Neonatal Society guideline on neonatal infections-diagnosis and treatment. Turk Pediatri Ars 2018;53(Suppl 1):88-100.
3. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. Arch Dis Child Fetal Neonatal Ed 2015;100:F257-63.
4. Shane AL, Sanchez PJ, Stoll BJ. Neonatal sepsis. Lancet. 2017;390(10104):1770-80.
5. Pammi M, Weisman LE. Late-onset sepsis in preterm infants: update on strategies for therapy and prevention. Expert Rev Anti Infect Ther. 2015;13:487-504.
6. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol 2010;37:421-38.
7. Omran A, Maaroof A, Saleh MH, et al. Salivary C-reactive protein, mean platelet volume and neutrophil lymphocyte ratio as diagnostic markers for neonatal sepsis. J Pediatr (Rio J) 2018;94:82-7.
8. Can E, Hamilcikan S, Can C. The Value of Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio for Detecting Early-onset Neonatal Sepsis. J Pediatr Hematol Oncol 2018;40:e229-e32.
9. Temel MT, Coskun ME, Akbayram S, et al. Association between neutrophil/lymphocyte ratio with ductus arteriosus patency in preterm newborns. Bratisl Lek Listy 2017;118:491-4.
10. Kurtul BE, Kabatas EU, Zenciroglu A, et al. Serum neutrophil-to-lymphocyte ratio in retinopathy of prematurity. J AAPOS 2015;19:327-31.
11. Raimondi F, Ferrara T, Maffucci R, et al. Neonatal sepsis: a difficult diagnostic challenge. Clin Biochem 2011;44:463-4.
12. Paolucci M, Landini MP, Sambri V. How can the microbiologist help in diagnosing neonatal sepsis? Int J Pediatr 2012;2012:120139.
13. Chauhan N, Tiwari S, Jain U. Potential biomarkers for effective screening of neonatal sepsis infections: An overview. Microb Pathog 2017;107:234-42.
14. Mussap M, Noto A, Cibecchini F, et al. The importance of biomarkers in neonatology. Semin Fetal Neonatal Med 2013;18:56-64.
15. Liu X, Shen Y, Wang H, et al. Prognostic Significance of Neutrophil-to-Lymphocyte Ratio in Patients with Sepsis: A Prospective Observational Study. Mediators Inflamm 2016;2016:8191254.
16. Hwang SY, Shin TG, Jo IJ, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. Am J Emerg Med 2017;35:234-9.
17. Ljungstrom L, Pernestig AK, Jacobsson G, et al. Diagnostic accuracy of procalcitonin, neutrophil-lymphocyte count ratio, C-reactive protein, and lactate in patients with suspected bacterial sepsis. PLoS One 2017;12:e0181704.
18. Meshaal MS, Nagi A, Eldamaty A, et al. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) as independent predictors of outcome in infective endocarditis (IE). Egypt Heart J 2019;71:13.
19. Kaplan M, Ates I, Oztas E, et al. A New Marker to Determine Prognosis of Acute Pancreatitis: PLR and NLR Combination. J Med Biochem 2018;37:21-30.
20. Tumturk A, Ozdemir MA, Per H, et al. Pediatric central nervous system tumors in the first 3 years of life: pre-operative mean platelet volume, neutrophil/lymphocyte count ratio, and white blood cell count correlate with the presence of a central nervous system tumor. Childs Nerv Syst 2017;33:233-8.
21. Karabulut B, Arcagok BC, Simsek A. Utility of the Platelet-to-Lymphocyte Ratio in Diagnosing and Predicting Treatment Success in Preterm Neonates with Patent Ductus Arteriosus. Fetal Pediatr Pathol 2019:1-10.
22. Alkan Ozdemir S, Arun Ozer E, Ilhan O, et al. Can neutrophil to lymphocyte ratio predict late-onset sepsis in preterm infants? J Clin Lab Anal 2018;32:e22338.
23. Sarkar S, Bhagat I, Hieber S, et al. Can neutrophil responses in very low birth weight infants predict the organisms responsible for late-onset bacterial or fungal sepsis? J Perinatol 2006;26:501-5.