Annals of Medical Research
DOI: 10.5455/annalsmedres.2018.07.134 2018;25(4)512-7

Original Article

Predictive value of serum neutrophil-to-lymphocyte ratio in bronchopulmonary dysplasia: A retrospective observational study

Ahmet Ozdemir

Education and Research Hospital, Clinic of Neonatology, Kayseri, Turkey

Abstract

Aim: Our objective in this study was to evaluate the predictive significance of serum neutrophil-to-lymphocyte ratio (NLR) in bronchopulmonary dysplasia (BPD) occurrence prediction.

Material and Methods: Under 32 weeks old preterm infants followed-up in our clinic between January 2015 and December 2017 were included in the study. Oxygen requirement continuing in postnatal 36th week was considered for BPD. Total blood count, C-reactive protein (CRP) and NLR in the first 24 hours of life were registered for both groups with and without BPD. The relation between BPD occurrence and risk factors was evaluated using univariate and multivariate logistic regression analysis.

Results: While 27 of 54 preterm infants included in the study were BPD patients, 27of them were preterm without BPD. Average NLR values of BPD and control group were 0.80±0.49 and 0.43±0.21 in order and a statistically significant difference was detected (p=0.001). NLR (OR: 0.042; 95% CI: 0.004-0.413;. p=0.001) was determined as independent risk factor for BPD occurrence in logistic regression analysis. NLR cut-off value was 0.64, sensitivity was 57.7%, specificity was 81.5% and the area below the curve was 0.737 (95% CI: 0.601-0.873) according to the ROC curve. A positively weak (r=0.26) statistically significant relation was detected among NLR and oxygen duration in Pearson correlation analysis (p=0.05).

Conclusion: NLR can be a promising device for predicting BPD patients in the first 24 hours. It was demonstrated that BPD could occur in premature infants with NLR>0.64.

Keywords: Bronchopulmonary Dysplasia; Lymphocyte; Neutrophil; Premature.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a condition in which is assisted by oxygen at least for 28 days, and in which respiration are required for preterm infants with gestational age below 32 weeks at postconceptional 36th week or at hospital discharge, and also for the infants with gestational age of 32 weeks and over on the 56th day or at hospital discharge (1). Beyond hyperoxia and/or immaturity inflammation play a part in the etiopathology of BPD (2). Pro-inflammatory cytokines such as IL-1 beta, and TNF-alpha initiate inflammation and increases tissue damage which constitute the basis of BPD (2).

While in whole blood count elevated neutrophil count indicates ongoing inflammation, diminished lymphocyte count is thought to be an indicator of poor prognosis. Therefore, the combination is considered to be predictive of an inflammatory condition (3).

In recent years the relationship between prematurity (4) and premature retinopathy (5,6), the problems of neonatal period, and neutrophil-lymphocyte ratio (NLR) has been proposed. However, there is no study on its relationship with BPD. In view of the role of inflammation in BPD pathogenesis this study has been done with the objective of assessing NLR, as a reliable prognostic marker of inflammation and suggesting the relationship between the development of BDP and NLR.

MATERIALS AND METHODS

Study population: The data for this study were collected from the premature hospitalized and followed-up in the

Received: 15.06.2018 **Accepted:** 20.07.2018 **Available online:** 30.07.2018
Corresponding Author: Ahmet Ozdemir, Kayseri City Hospital Neonatology Clinic, Kayseri, Turkey
E-mail: dr.ozdemir@yahoo.com

newborn clinic of Kayseri Training and Research Hospital between January 2015 and December 2017. The infants with birth weights below 2000 g and gestational ages under 32 weeks were included in the study. The patients born with hematologic diseases, those with sepsis proven with blood culture, and those diagnosed with necrotizing enterocolitis in stage 2-3 according to Modified Bell criteria were excluded.

This study has been approved by the Regional Ethic Committee of Kayseri Erciyes University, and done in compliance with the medical protocols and ethic-related principles of Helsinki Protocol.

Methods

The criteria set by the National Institute of Child Health and Human Development have been used for the classification of BPD (7). The gestational week of all the premature infants included in the study was below 32 weeks. Those who still required oxygen at postnatal 36th week were assigned to BPD group while those who did not meet this criterion were assigned to the control group.

The other variables related with BPD (gestational week, birth weight, way of delivery, multiple pregnancy, and sex) were recorded. The history, if any, of respiratory distress syndrome (RDS), surfactant requirement, intraventricular hemorrhage (IVH), premature retinopathy (ROP), premature membrane rupture, and maternal pre-eclampsia, was recorded as risk factors. The whole blood count parameters in the first 24 hours of life were recorded. In view of a potential infection risk and blood transfusion requirement during the clinical follow-up, blood samples were assessed within the first postpartum 24 hours. Serum neutrophil and lymphocyte count was done with Sysmex XN-100 biochemical analysis device.

Statistical Analysis: For data analysis use was made of SPSS v.22.0 for Windows (SPSS Inc, Chicago, IL). Of the continuous variables those with normal distribution were presented as the mean ± standard deviation, those without normal deviation as the median and interquartile percentage (IQRs). Categorical variables were presented as absolute numbers and percentage. Continuous variables were compared by means of t-test or Mann Whitney test. Categorical variables, however, were compared by means of chi-square Fisher exact test.

Univariate analysis was used for the assessment of potential risk factors in BPD such as NLR, RDS, mechanical ventilation, and surfactant therapy. Multivariate logistic analysis was done with a graded model to control all the variables and to predict the independent risk factors related with the presence of ROP.

The level of statistical significance was determined to be $p<0.05$. For each potential risk factor, corrected odds ratio (OR) 95% confidence interval (CI) were calculated. ROC analysis SPSS version 22.0 and easy ROC: a web-tool for ROC curve analysis (ver. 1.3) were used to determine the sensitivity and specificity of NLR , its optimal cut-off value in predicting BPD (8). Cut-off values were determined through Youden cut-off method.

RESULTS

This study has covered preterm infants with birth weights ranging from 710 to 1900 g and gestational ages from 25 to 32 weeks. Of these 54 infants, 27 had BPD, and the remaining 27 were without BDP but they were preterm. While 16(59%) of the patients with BPD were given steroid treatment, 11(41%) did not require steroid treatment. The main features of the premature infants in the BPD and control group have been presented in Table 1.

The mean gestational week of the infants diagnosed with BPD was 27.03±1.40 weeks (25-29 weeks), and their mean birth weight was 958.51±164.80 g(710-1400 g). The mean gestational week of the premature infants without BPD was 29.44±1.45 weeks (27-32 weeks), and their mean birth weight was 1491.55±218.31 g (800-1900 g). The mean pregnancy age and birth weight values in BPD group were lower. In addition, mechanical ventilation, oxygen therapy, RDS, surfactant need, ROP, and IVH were observed in greater degrees. The mean NLR values in BPD and control group were 0.80±0.49 and 0.43±0.21, respectively, and statistically significant difference was detected (p=0.001) (Table 1).

NLR values were seen to be (0.77±0.52) in the BPD patient group who had received steroid therapy, while it was (0.85±0.48) in the BPD patient group without steroid therapy. When compared with the values of the group without BPD (0.43±0.21), a significant increase was detected [p=0.004 (one-way ANOVA); Table 2, Figure 1].

However, no significant difference was detected in the other hemogram parameter (Table 2).

Logistic regression analysis has shown independent risk factors in BPD development as (OR: 0.042; 95% CI: 0.004-0.413; P=0.001) for NLR, (OR: 1.038; 95% CI: 1.015-1.048; P=0.001) for oxygen therapy duration, and (OR: 1.012; 95% CI: 1.002-1.021; P=0.015) for birth weight (Table 3).

Pearson correlation analysis revealed a poor (r=0.26), positive but statistically significant relationship between NRL and oxygen duration (p=0.05). The optimal cut-off value of NLR, determined to be an independent risk factor in BPD, was 0.64, its sensitivity was 57.7%, and its specificity was 81.5% with the area under the curve being 0.737 (%95 CI: 0.601-0.873) (Table 4).

The graphical and cumulative distribution of NLR values in BPD patients and the control group patients have been shown in Figure 2.

Table 1. Baseline clinical features and laboratory measurements of study subjects

Variable	BPD group (n=27)	Control group (n=27)	p value
Gestational age, weeks	27.03±1.40	29.44±1.45	<0.001
Birth weight, g	958.51±164.80	1491.55±218.31	<0.001
Sex (males)	13 (48.1%)	16 (59.3%)	0.41
Type of birth: C/S(%)	16 (59.3%)	20 (74.1%)	0.24
Multiple pregnancies (%)	4 (14.8%)	6 (22.2%)	0.48
WBC count (x10^3 µL)	4.56±0.89	5.97±1.14	0.50
Neutrophil count (x10^3 µL)	3.94±2.23	2.69±2.41	0.05
Lympocyte count (x10^3 µL)	5.36 (3.66-6.56)	4.88 (3.94-6.53)	0.76
Monocyte count (x10^3 µL)	0.82 (0.66-1.29)	0.63 (0.39-0.90)	0.05
Platelet count (x10^3 µL)	232.23±49.85	238.00±73.64	0.74
NLR	0.80±0.49	0.43±0.21	0.001
LMR	5.26 (4.35-6.97)	7.07 (4.40-13.94)	0.07
PLR	49.11 (31.53-65.95)	43.73 (27.11-66.24)	0.58
Hemoglobin g/dL	15.53±2.00	17.17±2.04	0.004
Hematocrit %	47.95±5.94	51.77±6.14	0.024
C-reactive protein (mg/dl)	3.36±1.51	3.12±1.89	0.87
Mechanical ventilation, n (%)	27 (100%)	19 (70.4%)	0.002
Mechanical ventilation, day	19.81±13.39	2.51±2.60	<0.001
Oxygen therapy, day	56.77±21.88	10.85±5.31	<0.001
RDS, n (%)	27 (100%)	20 (74.1%)	0.005
Surfactant therapy, n (%)	27 (100%)	18 (66.7%)	0.001
ROP, n (%)	16 (59.3%)	5 (18.5%)	0.002
IVH, n (%)	18 (66.7%)	9 (33.3%)	0.01

C/S, caesarean section; NLR, neutrophil/Lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; PLR: Platelet-to-lymphocyte ratio; RDS, respiratory distress syndrome; ROP: Prematurity of retinopathy; IVH, intraventricular hemorrhage; p<0.05, statistically significant

Table 2. Comparison of neutrophil, lymphocyte, monocyte, platelet, NLR, LMR, and PLR values among groups

Variable	BPD-group[a] (n=27)	BPD+steroid-group[b] (n=11)	BPD+steroid+ group[c] (n=16)	p value
Neutrophil count (x10^3 µL)	2.69±2.41	3.90±1.84	3.96±2.49	0.16
Lympocyte count (x10^3 µL)	4.88 (3.94-6.53)	4.83 (3.81-7.17)	5.36 (3.54-6.46)	0.75
Monocyte count (x10^3 µL)	0.63 (0.39-0.90)	0.76 (0.67-1.32)	0.96 (0.63-1.35)	0.38
Platelet count (x10^3 µL)	238.00±73.64	245.20±48.75	224.12±50.33	0.67
NLR	0.43±0.21x	0.85±0.48y	0.77±0.52y	0.004
LMR	7.07 (4.40-13.94)	5.20 (4.35-6.84)	5.58 (4.19-7.22)	0.25
PLR	43.73 (27.11-66.24)	53.87 (36.25-69.67)	49.11 (28.84-59.33)	0.75

The normal distribution was mean ± SD, the non-normal range was median and the 25 ± 75. It was expressed as a percentage. The different letters on the same line represent the difference; the same letters express the similarity between the groups. NLR: Neutrophil-to-lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; PLR: Platelet-to-lymphocyte ratio; BPD: Bronchopulmonary dysplasia
[a]Patients without BPD
[b]Patients with BPD who did not undergo steroid
[c]Patients with BPD who did undergo steroid

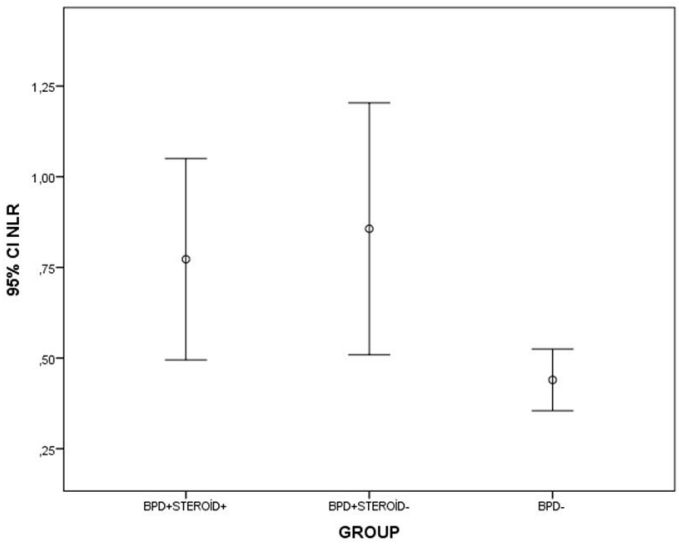

Figure 1. Comparison of neutrophil-to-lymphocyte ratio values among groups (p=0.01).

Table 3. Multivariate predictors of development of BPD in study population

Variable	Multivariate analysis	
	Odds ratio (95% CI)	P value
Gestational age	1.749 (0.492-6.211)	0.386
Birth weight	1.012 (1.002-1.021)	0.015
NLR	0.042 (0.004-0.413)	0.001
Oxygen therapy, day	1.038 (1.015-1.048)	0.001

95% CI: 95% confidence interval; NLR: Neutrophil-to-lymphocyte ratio; P<0.05, statistically significant

Table 4. Predictive value of NLR, LMR and PLR values in BPD patients

BPD group	Area Under the Curve		Diagnostic measures					
	AUC	p value	SEN (95%CI)	SPE (95%CI)	PPV	NPV	LR+	LR-
NLR (>0.64)	0.737 (0.601-0.873)	0.006	0.577 (0.369-0.766)	0.815 (0.619-0.937)	0.750 (0.526-0.878)	0.667 (0.462-0.871)	3.115 (1.322-7.340)	0.519 (0.320-0.842)
LMR (>4.3)	0.358 (0.077-0.206)	0.07	0.808 (0.606-0.934)	0.222 (0.086-0.423)	0.500 (0.248-0.772)	0.545 (0.306-0.755)	1.038 (0.788-1.368)	0.865 (0.301-2.492)
PLR (>48.69)	0.543 (0.080-0.385)	0.59	0.538 (0.334-0.734)	0.630 (0.424-0.806)	0.583 (0.377-0.768)	0.586 (0.378-0.776)	1.454 (0.792-2.668)	0.733 (0.442-1.216

BPD, Bronchopulmonary dysplasia; NLR, Neutrophil-to-lymphocyte ratio; LMR, Lymphocyte-to-monocyte ratio; PLR, Platelet-to-lymphocyte ratio; AUC, Area Under the Curve; SEN, Sensitivity; SPE, Specificity; PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR+, Positive Likelihood Ratio; LR-, Negative Likelihood Ratio

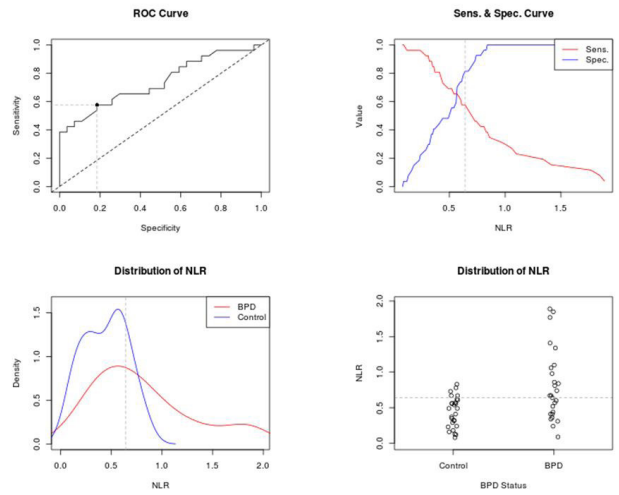

Figure 2. ROC curves of NLR values in BPD patients

DISCUSSION

As far as we know from the literature, this is the first study to address the relationship between BPD and NLR. We have found that NLR taken in the first 24 hours is correlated to BDP and is an important marker of BPD development.

Anti-oxidative stress, which results from the imbalance in the anti-oxidant and pro-oxidant systems, is one of the main risk factors in BDP development. It is important to know that hyperoxia is not the only factor to cause oxidative stress (9). The risk of oxidative stress development is high in premature infants due to their anti-oxidant defenses, which have not yet completed maturation, their increasing susceptibility, and their exposure to free irons (10). Oxidative stress gives rise to pulmonary development interrupted by the mechanisms involving the impairment of growth factor signals, extracellular matrix mechanism, cell proliferation, apoptosis, and vasculogenesis (11). In our study, the independent risk factors in BDP have been determined with logistic regression analysis as the duration of oxygen use, birth weight, and NLR.

Inflammation has an important place in BPD pathogenesis (1, 12). Chorioamnionitis is thought to be an important risk factor since it is systemic and initiates inflammation in the lungs in the intrauterine period (13). In addition, it is one of the most important causes of early membrane rupture and preterm births. Early diagnosis and treatment will reduce maternal and neonatal complication risk (14). The calculation of NLR in whole blood count intended

to show inflammation is an easy marker compared with the quantification of other cytokines such as TNF-α, IL-6, and IL-1β (15). In recent years, NLR, which quantifies the harmful effects of neutrophilia (an indicator of inflammation) and lymphopenia (a marker of physiologic stress), has been demonstrated to be a valuable marker in a multitude of studies assessing the patients with inflammation (6, 16-18). NLR shows also the balance between adaptive (lymphocytes) and natural (neutrophil) immunity. In previous studies, it has been demonstrated that NLR as a marker of inflammation is more valuable than leucocyte count (16, 19). Our study has shown the prognostic effect of NLR on BPD in logistic regression analysis. The inflammation involved in BPD pathogenesis considered, we have demonstrated the effect of NLR on the prediction of inflammation, consistent with the literature.

There are existing studies in the literature on patent ductus arteriosus (PDA) (20), Prematurity retinopathy (6), neonatal ischemic cerebral damage (21) with NLR in neonatal period. Temel et al.(20), have found a positive correlation between NLR and birth weight in patients with PDA and demonstrated that NLR can play a prognostic role in early diagnosis and clinical course of PDA in preterm infants. Our findings from this study, which are consistent with these studies, suggest the predictive importance of NLR in BDP patients. The diagnostic value of NLR has been propounded in a recent study on newborn with neonatal ischemic damage (21). In this study the importance NLR in terms of inflammation and immune response has been stressed. In the ROC analysis in our study the cut-off value of NLR for BPD has been found to be 0.64 with a sensitivity and specificity of 57.7% and 81.5%, respectively. In conclusion, we are of the opinion that NLR is a practical marker of inflammation in BPD patients.

In the study excepting the neonatal period, the role of NLR in predicting renal cortical defect and scar in patients with febrile urinary tract infection has been demonstrated (22). In another study it has been reported that NLR might arouse suspicion in early diagnosis of central nervous system tumors in the first three years of life (23). In the study by Mentis et al, it has been detected that NLR in cerebrospinal fluid can function as an additional biomarker for the differential diagnosis of bacterial and viral meningitis (24). The NLR inflammation relationship in this study is in agreement with our study.

Increases occur in NLR as a result of an increase in neutrophil count and a decrease in lymphocyte count. NLR represents the balance between the levels of neutrophil and lymphocytes in the body and is an indicator of systemic inflammation (25). The relationship of NLR with inflammatory cell activation, increase in acute phase reactant and plasma cytokine levels, oxidative stress, and pulmonary inflammatory infections like musculoskeletal dysfunction has been demonstrated (26-28). Supraphysiologic oxygen use is known to be related with BPD (29). Similarly, in our study oxygen use, the rate being put on mechanical ventilation, duration in mechanical ventilation, surfactant use, ROP, IVH, and NLR were detected in greater levels in the newborns with BPD than in control group. Additionally, a positive correlation was detected between NLR and oxygen use. Therefore, we think that NLR can be used as a prognostic marker in patients with oxygen-related BPD.

CONCLUSION

In conclusion, NLR is a biomarker easy to integrate into daily clinical routines since it is cost-effective and easy to calculate. We have found a significant positive relationship between NLR and BPD patients requiring steroid therapy in particular, which has led us to think that NLR can be a promising tool in predicting BPD patients within the first 24 hours BPD. We have demonstrated that BPD can develop in premature infants with NLR>0.64. The physicians working in neonatal intensive care units should bear in mind the complexity of knowing in what patient BPD will develop and that no biomarker can replace the preventive approach needed in assessing a BDP patient.

Financial Disclosure: There are no financial supports.

Ethical approval: This study was approved by the Regional Ethics Committee of Kayseri Erciyes University and conducted in accordance with the principles of the Helsinki Protocol.

REFERENCES

1. Bastug O, Ozdemir A, Gunes T. Bronkopulmoner Displazi: Güncel Yaklasımlar. Turkiye Klinikleri J Pediatric Sci 2015;11:26-37.
2. Ozdemir R, Yurttutan S, Talim B, et al. Colchicine protects against hyperoxic lung injury in neonatal rats. Neonatology 2012;102:265-9.
3. Imtiaz F, Shafique K, Mirza SS, et al. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med 2012;5:2.
4. Akkar OB, Sancakdar E, Karakus S, et al. Evaluation of maternal serum 25-Hydroxyvitamin D, paraoxonase 1 levels, and neutrophil-to-Lymphocyte ratio in spontaneous preterm birth. Med Sci Monit 2016;22:1238-43.
5. Hu YX, Xu XX, Shao Y, et al. The prognostic value of lymphocyte-to-monocyte ratio in retinopathy of prematurity. Int J Ophthalmol 2017;10:1716-21.
6. Kurtul BE, Kabatas EU, Zenciroglu A, et al. Serum neutrophil-to-lymphocyte ratio in retinopathy of prematurity. J AAPOS 2015;19:327-31.
7. Jobe AH, Steinhorn R. Can we define bronchopulmonary dysplasia? The J Pediatrics. 2017;188:19-23.
8. Goksuluk D, Korkmaz S, Zararsiz G, et al. ROC: an interactive web-tool for ROC curve analysis using R language environment. R J 2016;8:213-30.
9. Kalikkot Thekkeveedu R, Guaman MC, Shivanna B. Bronchopulmonary dysplasia: A review of pathogenesis and pathophysiology. Respir Med 2017;132:170-7.
10. Perrone S, Tataranno ML, Buonocore G. Oxidative stress and bronchopulmonary dysplasia. J clin Neonatol 2012;1:109-14.
11. Madurga A, Mižíková I, Ruiz-Camp J, et al. Recent advances in late lung development and the pathogenesis of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 2013;305:L893-905.
12. Speer CP. Inflammation and bronchopulmonary dysplasia: a continuing story. Semin Fetal Neonatal Med 2006;11:354-62.
13. Thomas W, Speer CP. Chorioamnionitis is essential in the evolution of bronchopulmonary dysplasia–the case in favour. Paediatric Respir Rev 2014;15:49-52.
14. Fahey JO. Clinical management of intra-amniotic infection and chorioamnionitis: a review of the literature. J Midwifery Womens Health 2008;53:227-35.

15. Turkmen K, Guney I, Yerlikaya FH, et al. The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail 2012;34:155-9.
16. Polat M, Bugdayci G, Kaya H, et al. Evaluation of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in Turkish patients with chronic plaque psoriasis. Acta Dermatovenerol Alp Pannonica Adriat 2017;26:97-100.
17. Solak B, Dikicier BS, Cosansu NC, et al. Neutrophil to lymphocyte ratio in patients with vitiligo. Postepy Dermatol Alergol 2017;34:468-70.
18. Velissaris D, Pantzaris ND, Bountouris P, et al. Correlation between neutrophil-to-lymphocyte ratio and severity scores in septic patients upon hospital admission. A series of 50 patients. Rom J Inteml Med 2018;9.
19. Guthrie GJ, Charles KA, Roxburgh CS, et al. The systemic inflammation-based neutrophil–lymphocyte ratio: experience in patients with cancer. Crit Rev Hematol 2013;88:218-30.
20. Temel M, Coskun M, Akbayram S, et al. Association between neutrophil/lymphocyte ratio with ductus arteriosus patency in preterm newborns. Bratisl Lek Listy 2017;118:491-4.
21. Povroznik JM, Engler-Chiurazzi EB, Nanavati T, et al. Absolute lymphocyte and neutrophil counts in neonatal ischemic brain injury. SAGE Open Med 2018;6:2050312117752613.
22. Lee JW, Park JS, Park KB, et al. Prediction of renal cortical defect and scar using neutrophil-to-lymphocyte ratio in children with febrile urinary tract infection. Nuklearmedizin 2017;56:109-14.
23. 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. Child's Nervous Syst 2017;33:233-8.
24. Mentis AF, Kyprianou M, Xirogianni A, et al. Neutrophil-to-lymphocyte ratio in the differential diagnosis of acute bacterial meningitis. Eur J Clin Microbiol Infect Dis 2016;35:397-403.
25. Zahorec R. Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lel Listy 2001;102:5-14.
26. Drews A, Pizzichini M, Pizzichini E, et al. Neutrophilic airway inflammation is a main feature of induced sputum in nonatopic asthmatic children. Allergy 2009;64:1597-601.
27. Furukawa T, Sakagami T, Koya T, et al. Characteristics of eosinophilic and non-eosinophilic asthma during treatment with inhaled corticosteroids. J Asthma 2015;52:417-22.
28. Nacaroglu HT, Erdem SB, Karaman S, et al. Can mean platelet volume and neutrophil-to-lymphocyte ratio be biomarkers of acute exacerbation of bronchiectasis in children? Cent Eur J Immunol 2017;42:358-62.
29. Principi N, Pietro GM, Esposito S. Bronchopulmonary dysplasia: clinical aspects and preventive and therapeutic strategies. J Transl Med 2018;16:36.