Original article

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Neutrophil to lymphocyte ratio, monocyte to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume as a predictor of sepsis mortality in children at Dr. Soetomo General Hospital

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Abstract

***Objective*: The purpose of this study was to analyze the neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), platelet to lymphocyte ratio (PLR), mean platelet volume (MPV) as a predictor for mortality in children with sepsis.**

***Design*: This was a prospective cohort study.**

***Setting*: In Pediatric Intensive Care Unit (PICU), Emergency Room, and pediatric ward at Dr. Soetomo General Hospital, Surabaya on March 1, 2020 to August 2020.**

***Patients and participants*: A total of 80 children consisted of 40 septic and 40 non-septic patients were included.**

***Measurement and results*: The leukocyte count in septic patients was not significantly higher than in non-septic patients with a p value>0.05. The number of neutrophils (12.99±7.35x10^3/mm^3 versus 9.12±6.67x10^3/mm^3) had a relevant and significantly higher increase in septic patients (p=0.014). The NLR value (8.99±6.73 versus 4.80±5.30; p=0.001) was higher in septic patients. The cut-off of NLR as a diagnostic marker for sepsis was 3.52 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and odds ratio (OR) of 82.50%, 47.50%, 61.11%, 73.08%, and 4.26 (p=0.004), respectively. The MLR, PLR, and MPV did not differ significantly between septic and non-septic patients. NLR values (11.61±7.39 versus 5.77±4.05; p=0.014) between survive and dead septic patients. The NLR cut-off 8.98 has sensitivity, specificity, PPV, NPV, and OR of 77.78%, 54.55%, 58.3%, 75%, and 4.20 (p=0.038), respectively, as a predictor for mortality. Patient with NLR more than 8.98 has a risk for mortality 4.20 times higher than those with a low or equal NLR value.**

***Conclusion*: NLR can be used as a predictor of mortality in children with sepsis.**

Key words: Neutrophil to lymphocyte ratio, monocyte to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume, sepsis mortality, children.

Introduction

Sepsis is a condition in which life-threatening organ dysfunction occurs due to immune dysregulation against infection. (1) The high mortality in sepsis is often caused by delay in diagnosis and treatment. (2) Several laboratory biomarkers have been used for the diagnosis of sepsis and as predictors of mortality. Neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), platelet to lymphocyte ratio (PLR), and mean platelet volume (MPV) are biomarkers that can be used to predict mortality outcomes in septic patients, but the role of these markers and cut-off values in pediatric patients is still rarely studied. (3,4) This study aims to analyze the NLR, MLR, PLR, and MPV as predictor factors for sepsis mortality in children.

Material and methods

This was a prospective observational study in pediatric patients with sepsis and without sepsis who were admitted to Pediatric Intensive Care Unit (PICU), Emergency Room, and pediatric ward at

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Dr. Soetomo General Hospital, Surabaya on March 1, 2020 to August 2020.

Subject enrolment
The sampling technique was carried out by consecutive sampling on subjects who met the inclusion criteria. The inclusion criteria was 1 month - 18 years old, sepsis, and their parents/guardians agreed to participate as the study sample and signed an informed consent. The exclusion criteria included blood cancer, platelet disorders, and splenectomy. Subjects met the dropout criteria if the patient went home at his own request.

Laboratory measurement
NLR is the ratio obtained from dividing the absolute value of neutrophils to the absolute value of lymphocytes. MLR is the ratio obtained from dividing the absolute value of monocytes to the absolute value of lymphocytes. PLR is the ratio obtained from dividing the absolute value of platelets to the absolute value of lymphocytes. MPV is the mean volume of platelets expressed in phento liters (fl). Complete blood count using the Sysmex XN 1000 and XN 3000 tools at the Clinical Pathology Laboratory of the Diagnostic Center Dr. Soetomo General Hospital, Surabaya.

Septic criteria
The diagnosis of sepsis was based on the presence of infection and organ dysfunction. Signs or evidence of ongoing infection: a) Clinical criteria: Fever (core temperature >38.5 °C or axillary temperature >37.9 °C) or hypothermia (core temperature <36 °C), tachycardia or bradycardia, tachypnea; b) Laboratory criteria: Leukocyte cut-off: 1 month-1 year: >17,500 or <5000/mm^3, 2-5 years: >15,500 or <6000/mm^3, 6-12 years: >13,500 or <4500/mm^3, 13-18 years: >11,000 or <4500/mm^3, or C-reactive protein (CRP) cut-off: 1.56-110 mg/l, or procalcitonin cut-off: 0.3-8.05 ng/ml, or culture presence of bacteria, and signs of organ dysfunction/failure: Pediatric Logistic Organ Dysfunction (PELOD)-2 score ≥11 for type A hospital, or ≥7 for type B or C hospital. (1) Subjects were classified as non-sepsis group if they did not meet the criteria for sepsis (no infection and organ dysfunction). The outcome that were assessed in this study were: alive (patients lived while they were and after undergoing therapy and were assessed for up to 28 days of observation) and death (patients died while being and after undergoing therapy and were assessed for up to 28 days of observation).

Data analysis
Data were analyzed using SPSS version 21.0. The differences in NLR, MLR, PLR, and MPV between survive and dead patients and septic and non-septic patients were tested using the independent sample T-test if the data were normally distributed, and using the Mann Whitney test if the data were not normally distributed. The level of significance was $p<0.05$ with 95% of confidence interval.

Study ethics
This study has been approved by the Health Research Ethics Committee Dr. Soetomo General Hospital, Surabaya with the ethical eligibility certificate number 1852/KEPK/III/2020.

Result
A total of 80 patients were enrolled in this study, 40 patients with sepsis and 40 patients without sepsis who met the inclusion and exclusion criteria. During 28 days of observation, 22 patients with sepsis died and 18 patients were alive, while in non-septic patients, all patients were alive. During the study, no patient dropped out. The basic and laboratory characteristics of the groups are listed in **Tables 1 and 2**.
The NLR value (8.99±6.73 versus 4.80±5.30; p=0.001) was higher in patients with sepsis, and was statistically significant. The values of MLR, PLR, and MPV did not differ significantly between septic and non-septic patients (p>0.05). The calculation of the values of NLR, MLR, PLR, and MPV is shown in **Table 3**.
Reference cut-off value of NLR as a diagnostic marker for sepsis was 3.52. This cut-off has a sensitivity, specificity, PPV, NPV, and OR of 82.50%, 47.50%, 61.11%, 73.08%, and 4.26, respectively (**Table 4**).
In sepsis patients, 22 patients died and 18 patients were alive. The mortality in the sepsis group was 45%. There was significant difference in the NLR (11.61±7.39 versus 5.77±4.05; p=0.014) between survive and dead septic patients, but no difference was found in MLR, PLR, and MPV between both groups (**Table 5**).
Multivariate analysis result showed that NLR was statistically significant for death outcomes in septic patients with adjusted odds ratio of 1.22 (p=0.05). Meanwhile, for the values of MLR, PLR, and MPV there were no significant effect on the occurrence of death (**Table 6**).
NLR in sepsis has an area under the curve (AUC) of 0.72 (95% CI 0.571-0.884). Meanwhile, MLR, PLR, and MPV were considered insignificant in

this study (**Figure 1**).
The NLR cut-off reference value of 8.98 has a sensitivity of 77.78%, a specificity of 54.55%, a PPV of 58.3%, and a NPV of 75% with OR of 4.20 (p=0.038) (**Table 7**).

Discussion

The leukocyte count in septic patients was not significantly higher than in non-septic patients. The number of neutrophils ($12.99\pm7.35x10^3/mm^3$ versus $9.12\pm6.67x10^3/mm^3$) had a higher increase in septic patients (p=0.014). No significant differences were found in hemoglobin levels, lymphocyte counts, monocytes, and platelets in patients with sepsis and non-sepsis. These results were in accordance with previous study which showed that there was no relationship between complete blood count and sepsis in children. (5)

Neutrophil to lymphocyte ratio in sepsis and non-sepsis

The results of this study indicate an increase in the NLR value in patients with sepsis compared to non-sepsis. The average NLR was 8.99. The increase in NLR occurs because of the mechanism responsible for the lymphocytopenia process in sepsis which involves the marginalization and redistribution of lymphocytes in the lymphatic system and the acceleration of the apoptotic process. The apoptotic process occurs when bacteria or products stimulate macrophages to release proapoptotic substances, such as tumor necrosis factor (TNF)-α, nitric oxide (NO), and glucocorticoids. This condition will then suppress lymphocyte production. (6) The increase in NLR occurs due to direct or indirect stimulation of the bone marrow which causes the number of neutrophils in the blood to increase. (7) The increase in neutrophils is caused by proinflammatory cytokines such as interleukin (IL)-6, IL-1, and TNF-α produced by macrophages and a decrease in the number of lymphocytes caused by increased secretion of glucocorticoid hormones which suppress lymphocyte production. (8)

Monocyte to lymphocyte ratio in sepsis and non-sepsis

There are very little literatures of MLR that we found in infection. In this study there was no significant MLR value difference between patients with sepsis and non-sepsis (0.6±0.38 in sepsis and 0.59±0.72 in those without sepsis, p=0.081). The same result as this study was a study conducted by Djordjevic, et al which stated that MLR value indicated negative blood culture in the group of peritonitis patients with AUC 0.586 (p=0.046), patients with negative blood cultures had a significantly higher MLR value. (3)

Platelet to lymphocyte ratio in sepsis and non-sepsis

The PLR is positively correlated with the presence of inflammation, so that it can be a useful biomarker to predict the severity of an inflammatory process that occurs. (9) This is in accordance with the study conducted by Meshaal, et al, which found a tendency to increase PLR in sepsis. (10) This is different from the results obtained in this study, namely that there was no significant difference between PLR values in patients with sepsis and non-sepsis. This difference can be caused by the timing of blood draws when the diagnosis of sepsis is different. The decrease in platelet count was statistically significant during the first 3 days of sepsis in gram-positive sepsis patients, for 4 days in gram-negative sepsis patients, and within 5 days in fungal sepsis patients (p<0.001). (11)

Mean platelet volume in sepsis and non-sepsis

Several studies have shown that MPV is increased in septic patients. Study by Catalm et al showed a significant increase in MPV in the sepsis group and reported that MPV may be a useful predictor for diagnosis. (12) MPV values were higher in patients with sepsis compared to those without. The results of this study indicated that there was no significant difference between MPV values in patients with sepsis and non-sepsis. (4) This difference relates to the appropriate time to examine the MPV in septic patients. The difference in blood draw time at the time of sepsis affects the MPV. (11)

Predictor value of neutrophil to lymphocyte ratio (NLR) in sepsis

The results of NLR prognostic test in this study also showed that the NLR cut-off reference value of 8.98 had a sensitivity of 77.78%, a specificity of 54.55%, a PPV of 55.33%, and an NPV of 75%. Patient with NLR more than 8.98 had a risk for mortality 4.20 times higher than those with a lower or equal NLR value. These results are similar to those of previous studies in that the outcome of an increase in NLR was predicted to be 61.1% and that a decrease in NLR was correlated with better survival. An increase in NLR>0.2 can predict mortality with a sensitivity of 89.2% and a specif-

icity of 61.1%. (13) Study by Yoldas, et al found that NLR of survive and dying patients was 2.06 (1.18-21.68) and 10.42 (2.85-48.2). NLR was significantly increased in patients who died compared with those who were alive ($p<0.001$). NLR value can predict mortality in the critically ill patient population. (14)

Conclussion

NLR can be used as a marker and predictor of mortality in children with sepsis. The cut-off value of NLR in children as a predictor of sepsis mortality in children with sepsis is 8.98 with a sensitivity of 77.78%, a specificity of 54.55%, with odds ratio of 4.20 ($p=0.038$).

Table 1. Basic characteristics of study subjects in the septic and non-septic groups

Variable	Sepsis (n=40)	Non-sepsis (n=40)	p value
Sex - Male (%) - Female (%)	 21 (52.50) 19 (47.50)	 24 (60.0) 16 (40.0)	0.449
Age (mean, SD)	6.20 (5.91)	5.35 (5.52)	0.640
PELOD score (mean, SD)	11.78 (2.20)	3.23 (1.85)	0.000

Legend: PELOD=Pediatric Logistic Organ Dysfunction; SD=standard deviation.

Table 2. Laboratory characteristics of study subjects in the septic and non-septic groups

Variable	Sepsis (n=40)		Non-sepsis (n=40)		p value
	Mean	SD	Mean	SD	
Hemoglobin (g/dl)	10.28	2.96	11.23	3.25	0.173
Leukocyte (cells/mm^3)	16531.75	9086.14	13723.00	8096.08	0.132
Neutrophil (cells/mm^3)	12999.25	7355.59	9121.00	6679.43	0.014
Lymphocyte (cells/mm^3)	2397.50	2696.88	2842.75	2119.80	0.067
Monocyte (cells/mm^3)	975.50	604.86	1174.50	997.77	0.305
Platelet (cells/mm^3)	267700.00	200657.92	317425.00	203754.74	0.305

Legend: SD=standard deviation.

Table 3. The values of NLR, MLR, PLR, and MPV in the septic and non-septic groups

Ratio	Sepsis (n=40)		Non-sepsis (n=40)		p value
	Mean	SD	Mean	SD	
NLR	8.99	6.73	4.80	5.30	0.001
MLR	0.60	0.38	0.59	0.72	0.081
PLR	214.85	252.73	157.78	163.48	0.814
MPV	10.20	1.18	9.92	1.18	0.218

Legend: NLR=neutrophil to lymphocyte ratio; MLR=monocyte to lymphocyte ratio; PLR=platelet to lymphocyte ratio; MPV=mean platelet volume; SD=standard deviation.

Table 4. NLR as a diagnostic marker for sepsis and non-sepsis

NLR	Sepsis (n, %)	Non-sepsis (n, %)	p value	OR
Low (≤3.52)	33 (61.1)	21 (52.5)	0.004	4.26
High (>3.52)	7 (26.9)	19 (47.5)		

Legend: NLR=neutrophil to lymphocyte ratio; OR=odds ratio.

Table 5. Differences in NLR, MLR, PLR, and MPV values in septic patients

	Outcome						p value
	Dead			Survive			
	Mean	n	SD	Mean	n	SD	
NLR	11.615	22	7.395936	5.776111	18	4.053548	0.014
MLR	0.660455	22	0.403136	0.527222	18	0.352484	0.527
PLR	269.0982	22	299.7929	148.5406	18	164.4645	0.157
MPV	10.14545	22	1.340834	10.26111	18	0.969013	0.761

Legend: NLR=neutrophil to lymphocyte ratio; MLR=monocyte to lymphocyte ratio; PLR=platelet to lymphocyte ratio; MPV=mean platelet volume; SD=standard deviation.

Table 6. The values of NLR, MLR, PLR, and MPV on life and death outcomes in septic patients

	β coefficient	Z score	p value	Adjusted OR
NLR	0.20	3.88	0.05	1.22
MLR	-0.99	0.38	0.54	0.37
PLR	0.00	0.71	0.40	1.00
MPV	-0.22	0.31	0.58	0.80

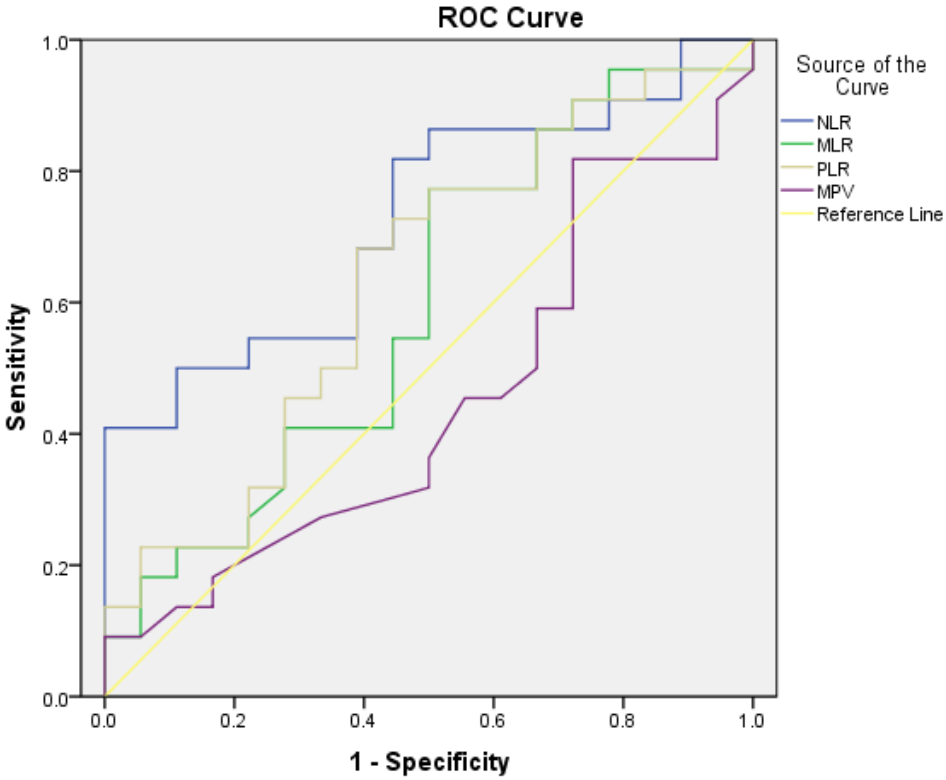
Legend: NLR=neutrophil to lymphocyte ratio; MLR=monocyte to lymphocyte ratio; PLR=platelet to lymphocyte ratio; MPV=mean platelet volume; OR=odds ratio.

Table 7. NLR as a predictor of mortality in septic patients

NLR	Outcome		p value	OR
	Survive, n (%)	Dead, n (%)		
Low (≤8.98)	14 (58.3%)	10 (41.7%)	0.038	4.20
High (>8.98)	4 (25.0%)	12 (75.0%)		

Legend: NLR=neutrophil to lymphocyte ratio; OR=odds ratio.

Figure 1. Area under the curve of NLR, MLR, PLR, and MPV in sepsis



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