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Strengthening the Capacity of Healthcare Providers through the Introduction of Hematological Indices as Sepsis Markers

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Abstract: *Sepsis is the most common cause of increased mortality and morbidity. Culture is a gold standard, but it is time-consuming and has a low positive rate. Currently, procalcitonin is a reliable biomarker for diagnosing and predicting sepsis, but the cost is high, and it is not always available in every laboratory facility. Objective: To examine neutrophil-lymphocyte count ratio(NLCR), platelet distribution width(PDW), and Mean Platelet Volume(MPV), which can be used as markers of sepsis compared with Procalcitonin(PCT). Method: This was a cross-sectional study of 40 adult patients who entered the Emergency Department. The study took place from May 2017 to June 2017. All patients had blood samples taken on the first day of treatment. Blood culture is used as the gold standard. PCT, NLRC, PDW, and MPV values were compared between patients with positive and negative blood cultures. Results: of 40 adult patients, 25 had positive blood culture, and 15 had negative results. The performance of PCT, NLRC, PDW, and MPV was significantly higher in patients with positive blood culture compared with the negative consequence AUC=0.915/0.768/0.756/0.733, P=<0.001/0.005/0.012/0.007,respectively).NLCR was found to have a better diagnostic efficiency for predicting sepsis, with greater sensitivity and accuracy than platelet indices. Spearman correlation test showed a significant correlation between NLCR and PCT levels in sepsis patients (rs=0.504; P=0.001). The combination of NLCR, PDW, and MPV demonstrated a good diagnostic performance (sensitivity 76%, specificity 66,7%), similar to procalcitonin (sensitivity 88%, specificity 66,7%). Conclusion: The increase of NLCR has an opportunity to be an indirect marker of increased PCT levels. Combining these three parameters could be a marker to distinguish sepsis from non-sepsis*

1. Introduction

Until now, sepsis has been a problem in the medical world and often causes death due to late diagnosis, so it is necessary to know and understand the existence of its signs to find it as early as possible. The ideal signs of sepsis diagnosis are: Very typical and sensitive; Easy to use; Fast and cheap; It is directly proportional to the turmoil. Sepsis is a condition in which an infection is found in the bloodstream that is specifically related to a serious illness. Blood cultures have been commonly used as a traditional approach in detecting sepsis. However,

blood cultures take a long time, so the diagnosis of sepsis is often too late to determine. In addition, the sensitivity of blood cultures decreases significantly if antibiotic therapy has been administered or when the cultured pathogen is a slow-growing, selective pathogen of the reproductive media. Therefore, other tests, such as complete blood and blood chemistry panels, are examined simultaneously or before the blood culture examination. There are currently some near-ideal signs of sepsis, such as Procalcitonin (PCT) and C-Reactive Protein (CRP). Still, they are often an obstacle, especially in developing countries, due to the high examinations cost. The research of Zhang et al found a new fact that the neutrophil-lymphocyte ratio, Platelet distribution width, has diagnostic value in sepsis patients. Sanci et al also examined the association of mean platelet volume in sepsis patients and found that MPV increased significantly in sepsis patients.

Platelet distribution width (PDW) and *Mean Platelet Volume* (MPV) are part of a routine complete blood test. PDW is an indicator of platelet size variation, which can indicate active platelet release. It has been shown that coagulation and platelet activation/hyperaggregation can occur in the early phases of sepsis. MPV measures the average size of platelets found in the blood. MPV is an index for inflammation, disease activity, and efficacy of anti-inflammatory treatment in several chronic inflammatory disorders, such as inflammatory bowel disease, rheumatoid arthritis, and ankylosing spondyloarthritis. High MPV levels are found in destructive thrombocytopenia, and low MPV levels in hypoproliferative thrombocytopenia. Increased platelet volume and size reflect a thrombotic and inflammatory environment. Thus, MPV is recommended as a marker of platelet function and activation. MPV and PDW are widely and routinely used in clinical practice worldwide. Some previous studies have also shown that higher MPV and increased PDW have been found in sepsis.

In addition, one of the physiological responses in the immune system against systemic inflammation is an increase in the number of neutrophils and a decrease in the number of lymphocytes. It is associated with changes in the dynamics and regulation of apoptosis in systemic inflammatory states compared to non-inflammatory states. The neutrophile/lymphocyte ratio is the absolute number of neutrophils divided by the absolute number of lymphocytes. The ratio of neutrophils to lymphocytes under physiological conditions is 2:1. In patients with sepsis, the ratio will increase.

Procalcitonin (PCT) is usually produced by C cells in the thyroid gland as a precursor to calcitonin. This PCT is present in low levels in the blood of healthy subjects. Recently, PCTs have been researched as an essential tool for early diagnosis of bacterial infections in clinical applications. Still, they are expensive and are not always available on every laboratory device.

The NLCR, PDW, and MPV parameters are simple tests at an affordable cost and part of a routine complete blood count. In this study, these parameters were compared with PCT to assess their performance as a marker of sepsis.

2. Method

Research design, patient, and clinical data. This research was carried out using the cross-cutting study method. The study population was all adult patients who were admitted to the emergency department of Saiful Anwar Hospital, Malang. The research lasted from May 2017 to June 2017. A total of 40 adult patients were included in this study. Clinical and laboratory data considered eligible, i.e., patients must meet the following inclusion criteria: i) Meet two or more SIRS criteria and there is suspicion or documented infection; ii) all blood samples from the studied patients were collected simultaneously during the same septic episode. The exclusion criteria are as follows: i) patients with hematological abnormalities such as hematological malignancies, metastases of malignancies that infuse the bone marrow, healing phase after bone marrow hyperplasia, or acute hemorrhage; ii) not meeting at least 2 SIRS criteria; iii) the sample was not collected simultaneously from the same study patient. Patients were classified into two groups based on the results of blood cultures. Patients with positive blood culture results are the positive group, and patients with negative blood culture results are grouped into the negative group. The characteristics of the study patients are attached in Table 1.

PCT, NLCR, PDW, MPV inspection. Simultaneously, blood samples from the same study patients were examined for Procalcitonin (PCT), Neutrophil-Lymphocyte Ratio (NLR), Platelet Distribution Width (PDW), and *Mean Platelet Volume* (MPV). PCT analysis was performed using a Cobas E411 *analyzer* (Roche Diagnostics, Basel, Switzerland). NLCR, PDW, and MPV were determined using the Sysmex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan).

Statistical analysis. Continuum variables and categorical variables are expressed as averages ± standard deviations. Data processing for normal distribution is done using the Shapiro-Wilk test. Comparison of continuum variables between the two groups was performed with an unpaired T-test for data with a normal distribution. At the same time, a Mann-Whitney test was used for abnormally distributed data. The *area under the curve* is calculated for each marker, and a *standard error* and a 95% confidence interval are determined. Correlation analysis (Spearman correlation) was performed between NLCR, PDW, MPV parameters, and PCT. $P<0.05$ was thought to indicate a statistically significant difference in all tests. Statistical

analysis was performed using SPSS version 17.

3. Results

Research Results.

Research population. Of the 40 adult patients in this study, 25 had positive blood culture results, and 15 had negative blood culture results. The demographic characteristics of the patients in the study are shown in Table 1. Statistical analysis showed no significant differences with respect to age or sex, according to the Shapiro-Wilk *test*. The statistical distribution of each marker tested in each group was analyzed using the Shapiro-Wilk test. In the following analysis, the unpaired T test was performed on parameters with normal distributions, while the Mann-Whitney test was performed on data with abnormal distributions.

Marker levels in the two study groups. The PCT, NLCR, PDW, and MPV concentrations were significantly higher in the positive group than in the negative group (Figure 1). The ROC curve was also calculated for marker analysis in patients with positive blood cultures compared with patients with negative blood cultures. The curved bottom region (AUC) is shown in Table 2. PCT has the widest AUC (AUC=0.915; 95%CI=0.814-1,000). The Spearman's correlation test showed a positive, meaningful correlation between NLCR and Procalcitonin (Table 3).

Table 1. Characteristics of adult patient groups

	Positive Group (n= 25)	Negative Group (n= 15)	P
Sex (Male: Female Ratio) **	14:11	8:7	Ns
Age (Year)(average±SD) Ɨ	54±19	50±16	Ns
SIRS criteria (average±elementary)			
Suhu (^{oC})*	36.5±0.7	36.8±0.6	ns
Heart Rate (beats/minute) Ɨ	101.1±18.2	93.8±15.1	ns
Breathing Rate (per minute) *	24.1±4.4	22.8±3.7	ns
Leucositol (x103/µL) *	18.2±13.5	15.6±9.6	ns
Immature Form (%)*	6.9±12.5	1.9±2.4	0.028
Other Hematological Parameters			
Rasio Neutrophil-Lymphocyte	22.4±17.9	13.6±22.6	0.005
(x103/ µL) *	231.9±129.8	348.1±182.6	0.038
Trombosit (x103/µL) Ɨ	10.7±0.9	9.9±0.9	0.007
Mean Platelet Volume (fL) Ɨ	12.5±2.6	10.5±1.8	0.012
Platelet Distribution Width (fL) *			
Procalcitonin*	29±36.5	0.6±0.6	0.000

**P value calculated using chi-square test; Ɨ P value is calculated using the unpaired T test; *P-values are calculated using the Mann-Whitney test

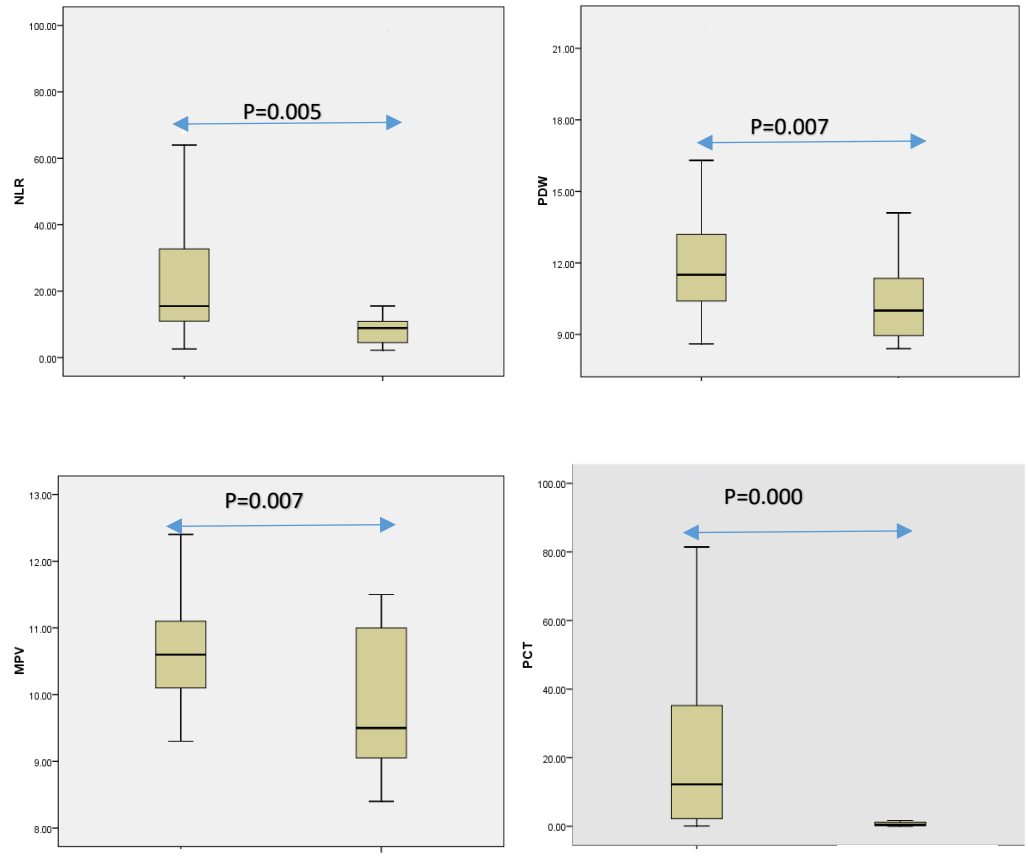

Figure 1. PCT, NLCR, PDW, and MPV in adult patient groups.

Box plots showed that PCT, NLCR, PDW, and MPV were higher in sepsis patients than in non-sepsis patients. Abbreviations: NLR, Neutrophil-lymphocyte ratio; PDW, Platelet distribution width; MPV, red platelet volume; PCT, Procalcitonin.

Table 2. Approximate diagnostic accuracy of the presence of sepsis

Parameter	AUC	95%CI	P
Procalcitonin	0.915	0.814-1.000	0.000
NLCR	0.768	0.609-0.927	0.005
PDW	0.756	0.589-0.923	0.007
MPV	0.733	0.559-0.908	0.015

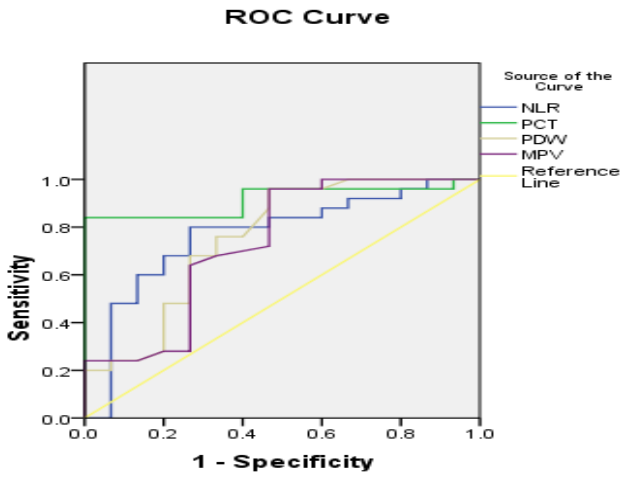

Table 3. Spearman's Correlation Test between NLCR, PDW, and MPV against Procalcitonin in Sepsis Patients

Korelasi Spearman's	Procalcitonin Adult patient group
NLCR	Rs=0.504, P=0.001
PDW	Rs=0.462, P=0.003
MPV	Rs=0.478, P=0.002

Table 4. Comparison of the outcome characteristics of sepsis markers and combination markers in sepsis prediction

Marker	Cut off	Sensitivitas(%)	Specificity(%)	Accuracy (%)	Positive predictive value(%) (95%CI)	Negative predictive value(%)(95%CI)
PCT	0,6	88	66,67	80	81,48 (67,95-90,13)	76,92(52,09-91,09)
NLCR	11,21	68	80	72,5	85(66,54-94,17)	60(44,53-73,70)
PDW	13,31	28	86.67	50	77,78(45,45-93,63)	41,94(34,52-49,74)
MPV	11,20	28	86,67	50	77,78(45,45-93,63)	41,94(34,52-49,74)
Kombinasi		76	66,67	72,5	79,17(64,25-88,93)	62,5(43,21-78,5)

PCT= procalcitonin (ng/ml), NLCR= Rasio neutrophil limfosit (103/µL), PDW= Platelet distribution width (fL), MPV= Mean Platelet Volume (fL), Kombinasi= NLCR+PDW+MPV, 95%CI= Interval 95% trust.

Discussion

Sepsis is a common cause of pain and death worldwide. Sepsis is a syndrome characterized by a systemic response to infection that can quickly progress to organ dysfunction and death. Sepsis is a normal reaction to infection, but it can last long due to bacteria, viruses, parasites, or fungi.[11] Early identification of sepsis-causing pathogens plays a vital role in appropriate treatment, and it has been reported that when proper treatment is initiated early, the rate of death caused by sepsis is reduced. On the other hand, delays in the diagnosis and treatment of sepsis will lead to rapidly developing circulatory failure, multiple organ dysfunction, and even death.[1] Signs of an ideal sepsis diagnosis are: Very distinctive and sensitive; Easy to use; Fast and cheap; Directly proportional to turmoil.[8]

There are currently some near-ideal signs of sepsis, such as Procalcitonin (PCT) and C-Reactive Protein (CRP). Still, they are often an obstacle, especially in developing countries, due to the high cost of screening and their not always being available in every healthcare facility.[8] Therefore, many studies have been developed on the hematology parameters for sepsis that can be useful in remote areas with limited laboratory facilities.

One of the physiological responses of the immune system to systemic inflammation is an increase in the number of neutrophils as well as a decrease in the number of lymphocytes. This is due to changes in the dynamics and regulation of apoptosis in the systemic inflammatory state compared to the non-inflammatory state. In studies of the immune response to inflammatory processes, in the group of patients given endotoxemia, after 4–6 hours, there will be a decrease in lymphocyte count by about 85% and neutrophils increase by about 300%. Delaying the neutrophil apoptosis process will prolong the function of neutrophils in the inflammatory process and prolong the process of toxic metabolic elaboration. On the other hand, an increase in lymphocyte apoptosis will result in a decrease in inflammatory and immunosuppressive [effectors.4] The number of neutrophils and lymphocytes is considered a parameter in a complete blood test routinely performed in the laboratory. For this reason, many researchers research this simple parameter [11]

In line with previous research, this study also found that procalcitonin showed significant results in distinguishing sepsis and non-sepsis patients compared to other markers, with the widest AUC of 0.915 (95% confidence interval: 0.814-1,000). In addition, it was found that the lymphocyte to neutrophil ratio ranks second in the order of the widest AUC (Table 3). This is in line with the study of Zhang et al, which found an AUC of 0.829 (95% confidence interval: 0.754-0.905) for procalcitonin and an AUC of 0.718 (95% confidence interval: 0.625-0.811) for neutrophil-lymphocyte ratio.[1]

Guclu et al also examined the association of platelet index with sepsis. Compared to controls, there was a decrease in platelets and an increase in MPV and PDW in sepsis patients. This condition is caused by the production of many cytokines and bone marrow suppression in sepsis patients. It has been shown that coagulation and activation/hypercoagulation can occur in the early phases of sepsis. To obtain a wider surface, the platelet changes its discoid shape to a spherical shape during the activation phase. At the same time, pseudopodia formation occurs. Platelets with an increase in the number and size of pseudopodia will affect PDW. MPV is related to platelet activation and function. Decreased platelets in severe sepsis explain the increase in MPV and PDW levels.[2] This is in accordance with the results of this study, which found that MPV and PDW are significant parameters in sepsis patients. This parameter was higher in sepsis patients than *non-sepsis* patients (PDW and MPV, p 0.007 each).

MPV describes both proinflammatory and thrombotic conditions, in which many cytokines are involved. Bacterial infections can trigger a variety of inflammatory cytokines, including procalcitonin, and procalcitonin itself increases leukocyte-derived cytokines. According to Cho et al. 's research, MPV increases in line with an increase in procalcitonin due to the rise in platelet volume, which is also caused by cytokine production and the release of bacterial endotoxins.[7] These results are consistent with this study, which also found a significant correlation between PDW and MPV against procalcitonin (Table 5). In addition, a significant positive correlation was also found between the neutrophil-lymphocyte ratio and procalcitonin, which was in accordance with the results of a study by Nurdani A (2016), who found a significantly strong correlation between NLCR and procalcitonin.[12] The cut-off value of procalcitonin was 0.6 ng/mL, with a sensitivity of 88% and a specificity of 66.67%. This is close to the procalcitonin cut-off value commonly used in diagnosing sepsis, which is 0.5 ng/mL, with a sensitivity of 76% and a specificity of 69%.[12] The combination of NLCR, PDW, and MPV results in sensitivity and specificity close to procalcitonin values, so it can be considered a marker to distinguish bacteremia from non-bacteremia.

4. Conclusion

PCT performance was found to be the most significant among other markers. There is a tendency to increase PCT in line with the increase in NLCR in sepsis patients, so this increase in parameters can be used as an indirect marker of an increase in PCT levels. NLCR, PDW, and MPV can be easily performed using automated hematology analysis at a low cost. The combination of these three parameters results in a sensitivity and specificity close to the value of procalcitonin, so it can be considered a marker to distinguish bacteremia from non-

bacteremia. This study has limitations in terms of the number of samples. Further diagnostic test research with larger sample numbers is needed to ensure these parameters' readiness in sepsis management protocols.

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