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THE DIFFERENCE BETWEEN ABSOLUTE NEUTROPHILS,NEUTROPHIL/ LYMPHOCYTE RATIO AND PLATELET/LYMPHOCYTE RATIO IN NORMAL, NSTEMI, STEMI PATIENTS

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ABSTRACT

Acute Coronary Syndrome (ACS) is a Non-ST-Elevation Myocardial Infarct (NSTEMI) and ST-Elevation Myocardial Infarct (STEMI) caused by atherosclerosis vascular, endothelial dysfunction as an acute inflammatory response. Absolute Neutrophils (AN), Neutrophil/Lymphocyte Ratio (NLR), and Platelet/Lymphocyte Ratio (PLR) is a systemic inflammation marker in inflammatory diseases. The research purpose was to compare AN, NLR, and PLR as an inflammatory marker in normal patients and, NSTEMI, STEMI. A cross-sectional observational study of 101 ACS patients at the Dr. Kariadi Hospital, divided into three groups (normal, NSTEMI, and STEMI). Neutrophil and lymphocyte were counted manually. Leukocyte and platelet value were determined by hematology analyzer. Data were analyzed with the Kruskal-Wallis test followed by post hoc Mann-Whitney. There was a difference in AN, NLR value between STEMI and NSTEMI compared to normal patients. There was no significant difference between PLR value.

Key words: Absolute neutrophils, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, NSTEMI, STEMI

INTRODUCTION

Cardiovascular disease is the leading cause of death and abnormality in the world caused by vascular atherosclerosis. Cardiovascular disease, including disease Coronary Heart Disease (CHD) is a chronic disease and belonging to non-transmitted diseases. It is one of heart and blood vessel disease that cause of death and abnormality in the world caused by vascular atherosclerosis, based on Riskesdas 2013 has a prevalence based on a physician's diagnosis of 0.5% and based on symptoms or a doctor's diagnosis of 1.5%.[1-3]

Coronary heart disease commonly known as Acute Coronary Syndrome (ACS) is a collection of clinical symptoms caused by acute myocardial ischemia, has high morbidity and mortality resulting in rapid detection and prognosis estimates in patients with chest pain. Acute coronary syndrome is divided into two main categories based on a change in the Electrocardiogram (ECG) with ST-Elevation of Myocardial Infarction (STEMI) and without ST-Elevation of Acute Coronary Syndrome consist of Non-ST-Elevation Myocardial Infarction (NSTEMI) and Unstable Angina Pectoris (UAP). The differences between NSTEMI and UAP showed increased levels of myocyte necrosis markers and ST-depression (Q wave myocardial infarction).[4,5]

According to the American College of Cardiology ACS criteria, are increased enzymes (CKMB, troponin I or troponin T) with signs of ECG changes leading to ischemia.[5] Blockage of more than 75% of the arterial lumen in coronary arteries and branches called acute myocardial infarction results in irreversible ischemia and myocardial necrosis due to reduced perfusion, caused by coronary artery obstruction from acute atherosclerosis and thrombotic processes. The causes of atherosclerosis include coronary artery spasm, embolism, periarteritis, other arterial inflammatory diseases, aneurysms, congenital coronary artery disease, increased blood viscosity (polycythemia vera) and myocardial infarction with normal coronaries.[6]

The presence of unstable coronary artery blockage leads to an acute/chronic inflammatory state associated with leukocytes where mononuclear cells (monocytes/macrophages, T-lymphocytes) can prevent plaque on unstable coronary arteries accompanied by neutrophil activation and increased concentrations of proinflammatory cytokines such as TNF-α, IL-6, CRP. The number of leukocytes in the peripheral blood may be used as one of the potential biomarkers useful for predicting cardiovascular risk, as an efficient and accessible inflammatory state in

the blood.[7]

Low lymphocyte counts are associated with poor cardiovascular conditions in patients with ACS and Chronic Heart Failure (CHF). Lymphocytopenia is a common finding in chronic inflammatory states because of increased lymphocyte apoptosis. Lymphocytes as an immune response, prevent neutrophils from causing damage to an inflammatory reaction. The lymphocyte count indicates an early marker of physiological stress and systemic inflammation.[8,9] Neutrophil/Lymphocyte Ratio (NLR) is a marker of systemic inflammation that has a predictive value on cardiovascular risk as in diabetes mellitus. Acute coronary syndrome, other inflammatory diseases. Neutrophil/lymphocyte ratio is a systemic inflammatory parameter that can predict life expectancy after Percutaneous Coronary Intervention (PCI), Coronary Artery Bypass Grafting (CABG).[10]

Increase of megakaryocytes proliferation and relative thrombocytosis are two results of the ongoing inflammatory state in the body, which result in a prothrombotic condition. Platelet/lymphocyte ratio value correlated with the amount of atherosclerotic coronary burden in CAD patient and also healthy adults with increased platelet counts have an augmented risk of thrombotic complication. The circulating platelets may contribute to the initiation of atheromatous plaque formation and triggers its complication. Platelet/lymphocyte ratio is a new prognostic marker and a marker of systemic inflammation that can provide an overview of the pathway of aggregation and inflammation in ACS.[8,9]

Platelets release a wide range of inflammatory mediators from intracellular stores, such as platelet factor 4, β-thromboglobulin, and CD40 ligand. Platelets may also induce the expression of such substances in leucocytes and endothelial cells. Platelets may respond to inflammatory mediators produced by the cells. Local inflammation and activated platelets promote the recruitment of more platelets via thrombopoietin and cytokines. Then an elevated platelet count may promote a heightened inflammatory state.[10]

Based on the above observations, the researchers wanted to compare the total number of neutrophils, the ratio of neutrophils/lymphocytes and platelets/lymphocytes in normal patients, STEMI and NSTEMI in the Dr. Kariadi General Hospital.

METHODS

This was analytical observational research with a cross-sectional approach conducted in February 2014 - February 2015 in the Dr. Kariadi General Hospital, Semarang. The study data were taken from the medical records of patients diagnosed as normal patients, NSTEMI, and STEMI, according to the International Classification of Diseases (ICD) 10, who was hospitalized.

Inclusion criteria were STEMI, NSTEMI, while patients with acute or chronic infections, bleeding, postoperative, allergic or parasitic infections, patients with malignancy were excluded from the study.

Leukocyte examination, platelets were examined by hematology analyzer using flow cytometry method, while the calculation of absolute neutrophils obtained from hematology instruments. Neutrophils/lymphocytes ratio was obtained from the percentage neutrophil type count divided by the percentage of lymphocyte type count. Platelet/lymphocyte ratio was obtained from the number of platelets divided by the percentage of lymphocyte count times the number of leukocytes.

Data were processed by statistical software for distribution, frequency, and average. Normality test by Shapiro-Wilk, different test by one way ANOVA, for abnormal data distribution test conducted using Kruskal Wallis and followed by Mann-Whitney test for post hoc analysis. This research was approved by Health Research Ethical Committee Medical Faculty of Diponegoro University/Dr. Kariadi General Hospital No. 30/EC/FK-RSDK/2015.

RESULTS AND DISCUSSION

The subjects consisted of 101 patients, consisting of 38 normal patients, 24 NSTEMI patients, and 35 STEMI patients. The youngest age was 43 years, and the oldest was 81 years old. Data on the characteristics of the research subjects as a whole can be seen in Table 1. The normal distribution of data was shown with mean±SD, and data with abnormal distribution was shown with median (minimum;maximum).

The subjects of the study were categorized by age, Hb, Ht, leukocytes, platelets, neutrophils, lymphocytes, PLR, NLR, Troponin, CKMB, as shown in Table 1 and Figure 1. In Table 2 and Figure 2 the hematologic characteristics of Hb, leukocyte, platelet, neutrophil, lymphocyte, absolute neutrophils, PLR, NLR were divided into three, normal study groups, STEMI, and NSTEMI.

Characteristics of neutrophils of normal patients 55.289±6.908, NSTEMI 70.478±11.95, STEMI 73.125±8.346 (Table 2). There was an increase in neutrophils between normal and sick patients, on the

Table 1. Overall patient characteristics

Characteristic	Median (min;max)	Mean±SD
Age	-	58.64 ± 10.123
Hb	-	13,808 ± 2.126
Ht	-	40.42 ± 6.232
Leukocyte	-	11.967 ± 3.692
Platelet	241,65 (87;1088)	-
Neutrophil	-	71.886 ± 10.649
Lymphocyte	18 (4;77)	-
PLR	0.124 (0.04;1.78)	-
NLR	3.28 (0.86;23.5)	-
Troponin	1.275 (0.01;24.03)	-
CKMB	35.5 (11;468)	-

SD: Standard Deviation

Table 2. Characteristics of normal patients, NSTEMI, STEMI

Characteristics	Normal Patient		NSTEMI		STEMI	
	Median (min; max)	Mean±SD	Median (min; max)	Mean±SD	Median (min; max)	Mean±SD
Hb	-	14.46±1.55	-	14.48±1.66	-	13.123±2.29
Leukocyte	7.7 (4.44;15)	-	-	11.058±3.207	-	12.162±4.104
Platelet	-	277.87±70.86	258,8 (150;1088)	-	-	236,79±88.98
Neutrophil	-	55.289±6.908	-	70.478±11.95	-	73.125±8.346
Limphocyte	-	35.868±7.367	19 (4;77)	-	-	15.84±5.837
Neutrophil absolut	4119,10 (2440;9216)	-	-	7925,2±3198,14	-	9.101±3794,23
NLR	-	1.64±0.548	3.75 (0.86;23.5)	-	4.54 (2.03;12.43)	-
PLR	0.099 (0.055;0.21)	-	0.133 (0.04;1.78)	-	-	0.148±0.066)

SD: Standard Deviation

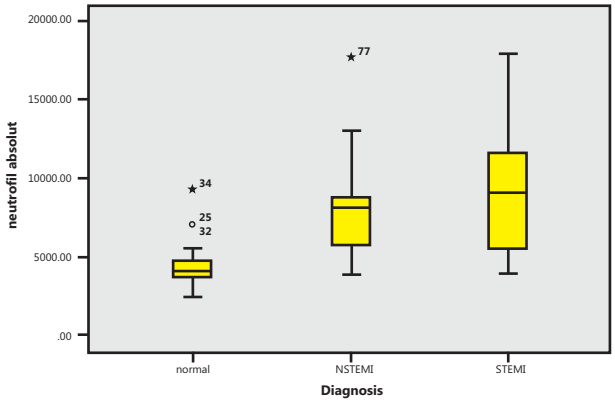

Figure 1. Absolute neutrophil data distribution of the normal patient group, NSTEMI, and STEMI

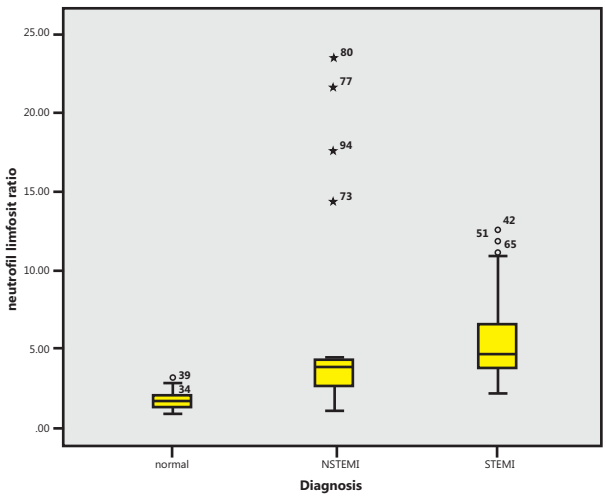

Figure 2. Distribution of NLR data in the normal patient group, NSTEMI, and STEMI

difference test, there was a significant difference in absolute neutrophils, normal patients-STEMI and normal patients-NSTEMI with p=0.000, whereas absolute neutrophils STEMI-NSTEMI was not significant (p=0.232). Characteristics of platelet and lymphocytes in this study showed that the distribution of data was not the same. There is a normal and abnormal, but it could be seen that there was a decrease in the number of both parameters in patients NSTEMI and STEMI compared to normal patients in Table 2.

Table 3. Absolute neutrophil differences test results in all three groups of patients

Characteristic	P absolute neutrophil	P NLR	P PLR
Normal Patients-STEMI	0.000*	0.000*	0.051
Normal Patients-NSTEMI	0.000*	0.000*	0.039
STEMI-NSTEMI	0.232	0.047	0.720
	p-test Kruskal-Wallis in three groups (p<0.001), significant	P-test Kruskal-Wallis in three groups (p<0.001), significant	p-test Kruskal-Wallis in three groups (p=0.056)

* Mann-Whitney's significant post hoc

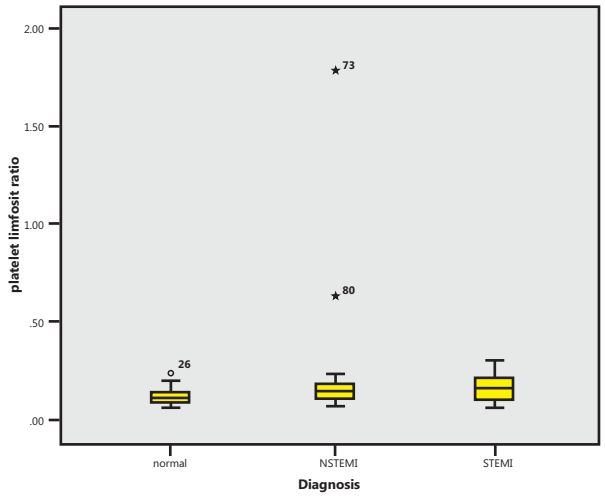

Figure 3. Distribution of PLR data in normal patients group, NSTEMI and STEMI

The absolute neutrophils, NLR, and PLR data normality tests were performed using the Shapiro-Wilk test since the distribution of the data were not normally distributed, Kruskal-Wallis test was followed by the Mann-Whitney post hoc test. The results of the absolute neutrophils difference test, NLR and PLR are shown in Table 3 and Figure 3.

Based on Kruskal-Wallis test a significant difference in absolute neutrophils and NLR value (p <0.001) was shown, followed by post hoc test of Mann-Whitney absolute neutrophils and NLR obtained a difference between normal patients with NSTEMI and also there was a difference between normal patients with STEMI but there was no difference between the NSTEMI group and STEMI. As for Kruskal-Wallis PLR test was (p=0.056). There was no difference between PLR in normal patients with NSTEMI, normal patients with STEMI and NSTEMI patients with STEMI.

Increased neutrophils with decreased lymphocyte counts led to higher NLR ratios in this study in NSTEMI and STEMI patients than in normal patients, but differed in PLR ratio, which had higher values in NSTEMI and STEMI than in normal patients. While on the different NLR test there was a significant difference (p=0.000) between normal patients and STEMI, also between normal and NSTEMI patients, but there was no NLR difference between STEMI and NSTEMI patients. In contrast to PLR difference test, there was no difference between the three groups of normal patients, STEMI and NSTEMI.

This study was consistent with Kazi *et al*. NLR was found to be normal in noncardiac patients but there was a significant increase in myocardial infarction patients, in particular there were significant differences in STEMI and NSTEMI (p <0.001). Different from that of Ghaffari *et al*. showing total neutrophils and neutrophil/lymphocyte ratios in postpartum STEMI patients in the hospital who were found to have no significant association with mortality but had a direct relationship with the frequency of cardiac failure and the development of ventricular arrhythmia on the first day, while for the total neutrophil count in this study it was the best prediction for death and heart failure.[9-14]

Lymphocyte neutrophils ratio as a short-term predictor in death of patients with acute coronary disease in which neutrophils provide a biochemical response such as the release of arachidonic acid metabolism and platelet aggregation factors, cytotoxic oxygen as free radicals and hydrolytic enzymes such as myeloperoxidase, elastase, and phosphatase acid. The incidence of myocardial infarction was accompanied by an increase in neutrophils, in which neutrophilia signified the presence of maladaptation, i.e. leukocytes in the circulation and platelet aggregation in acute coronary syndromes leading to vascular obstruction and widening of the infarction region. Decreased CD4 cell count and CD4/CD8 ratio were also observed in acute coronary patients.[8]

Increased number of neutrophils resulting from activation of neutrophils due to cytokines, some

chemoathractants are released during the inflammatory process, when activated neutrophils occur metabolic activation, increased cell activity, the release of granules in the blood and tissues leading to increased inflammatory responses as well as oxidative stress. The two components released during neutrophil activation in the body are elastase and lactoferrin. Elastase is stored in the azurophilic neutrophil granules which have proteolytic and bactericidal effects. Lactoferrin is a glycoprotein stored as specific granules or the second granules of neutrophils that contribute to the immune response and is associated with the antimicrobial activity to anti-inflammatory effects such as TNF-α and IL-6. In addition, neutrophils also act as a marker of Systemic Inflammatory Response (SIR) in adults.[5-11]

Zazula *et al*. showed that STEMI patients had lower lymphocyte counts than NSTEMI patients. This was associated with increased cortisol resulting in lymphocyte apoptosis. Lymphocytes are the first physiological response to stress and mediators of inflammatory processes in adults. In the case of inflammation, the number of lymphocytes decreases due to increased apoptosis of lymphocytes. Production of leukocytes in the bone marrow is shifted towards increased neutrophils and decreased lymphocytes.[15,16]

The inflammatory state will cause a proliferative increase in megakaryocytes and cause reactive platelets. The inflammatory state plays an important role in atherosclerosis of blood vessels. Platelet activation produces inflammatory substances from the endothelial and leukocytes that cause monocyte and transmigration adhesion, increased inflammatory processes, and atherosclerotic plaque. Platelets play a role in the pathogenesis of ACS with the combined form of fibrin platelets. Temiz *et al*. showed that enhancement of platelets could alter the viscosity and increase inflammation. High platelets are associated with levels of CRP and fibrinogen.[17]

Platelet Lymphocyte Ratio (PLR) plays an important role in the immune response where cytokine release increases the severity of ischemia and end-stage organ damage. According to Demirag *et al*. the high PLR in the STEMI population showed a poor prognosis compared to low PLR and there was a relationship between high PLR and NLR with mortality and life expectancy in the postoperative period of cardiovascular risk patients.[9,14] Oylumlu *et al*. showed that high PLR caused an increase in the death of NSTEMI patients and as a predictor of death of ACS patients.[12]

This study was limited to medical records, and prospective studies are recommended.

CONCLUSIONS AND SUGGESTIONS

There were differences between the absolute neutrophils and neutrophil/lymphocyte ratio between normal patients with NSTEMI and between normal patients with STEMI. There was no difference in platelet/lymphocyte ratio between the three groups of above patients, so absolute neutrophils and NLR may serve as independent factors to describe disease prognosis, this should be confirmed by other prospective studies.

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