

Correlation between Leucocyte Count, Neutrophil Lymphocyte Ratio (NLR), C-reactive Protein (CRP) and Coronary Artery Stenosis Degree on Stable Coronary Artery Disease

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Correlation between Leucocyte Count, Neutrophil Lymphocyte Ratio (NLR), C-reactive Protein (CRP) and Coronary Artery Stenosis Degree on Stable Coronary Artery Disease



Edward Kurnia Setiawan L,* Purwanto Adhipireno, Imam Budiwiyo

ABSTRACT

Background: Inflammation plays an important role in stable coronary artery disease (SCAD). Leucocyte count, NLR and CRP are markers of atherogenic and inflammatory processes in the atherosclerosis formation. The stenosis degree indicates a visual evaluation of the percentage reduction of coronary diameter compare with normal coronary artery.

Objective: to determine the correlation of leucocyte count, NLR and CRP with the stenosis degree in SCAD.

Methods: Analytic-descriptive with cross sectional study in 35 patients with SCAD at Kariadi Hospital. The study was conducted during March-June 2018. Leucocyte count was measured by hematology analyzer.

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NLR was calculated as the ratio of absolute neutrophil cell count to absolute lymphocyte cell count. CRP was measured by i-chroma reader. Statistical analysis used Spearman test. $p < 0.05$ was significance.

Result: Mean \pm SD leucocyte count, NLR, CRP respectively were $7,54 \pm 2,03/\mu\text{l}$; $2,10 \pm 0,93$ and $2,23 \pm 1,68$ mg/L. Correlation between leucocyte count, NLR, CRP and stenosis degree, respectively were ($r = 0,189$; $p = 0,277$); ($r = 0,593$; $p = 0,000$); ($r = -0,112$, $p = 0,521$).

Conclusion: There are no significant correlation between leucocyte count and CRP with stenosis degree in SCAD. There is strong positive correlation between NLR and stenosis degree in SCAD that can be used as evaluation marker for high risk patient with SCAD.

Keywords: Leucocyte count, NLR, CRP, stenosis degree, stable coronary heart disease

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INTRODUCTION

Cardiovascular disease is currently one of the main and first causes of death in both developed and developing countries.^{1,2} WHO estimates that there are around 20 million deaths from cardiovascular disease in 2015. This fact shows that cardiovascular disease will continue to dominate the numbers of future death. The prevalence of coronary heart disease diagnosed by doctors in Indonesia according to the Basic Health Research of the Health Research and Development Agency in 2013 was 0.5% or estimated at around 883,447 people.³

Coronary heart disease is a cardiovascular disease caused by narrowing the coronary arteries due to atherosclerosis. One manifestation of coronary heart disease is stable coronary heart disease. Stable coronary heart disease refers to patients who have been diagnosed with coronary heart disease or who are suspected of having coronary heart disease with constant acute symptomatic status.⁴⁻⁶ Dyslipidemia is a risk factor for stable coronary heart disease because dyslipidemia plays an important role in the process of formation of atherosclerosis in blood vessels that can lead to stable coronary heart disease.^{7,8} Stable coronary heart disease is also

known as chronic inflammation process. Previous studies have shown signs of inflammation are associated with the severity of stable coronary heart disease and worsening cardiovascular outcomes.⁹⁻¹¹

Increasing numbers of leukocytes indicate infection and inflammation that contribute to atherogenesis, development of atherosclerotic plaques, rupture and thrombosis. In the last decade, the researchers studied the relationship between atherosclerosis, coronary heart disease and acute coronary syndrome (ACS) with leukocytes, because leukocyte examination is easy and inexpensive. The study by Tahir *et al.* (2009) proved that leukocyte counts as independent predictors of short-term and long-term mortality in patients with ACS. Leukocyte count is also strongly positively correlated with coronary heart disease risk factors, cardiac biomarkers and CRP.¹²

The process of atherogenesis involves neutrophils as a marker of non-specific inflammation and lymphocytes as a marker of regulation.^{12,13} Neutrophil lymphocyte ratio (NLR) is an independent prognostic factor in coronary heart disease which is also influenced by hypercholesterolemia,

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metabolic syndrome, diabetes and hypertension. Neutrophil lymphocyte ratio can also be a predictor of mortality in cardiovascular disease.¹⁴ Previous studies have shown a correlation between NLR and stenosis severity in stable coronary heart disease based on results of coronary angiography.¹⁵ There were significant differences in NLR in groups with higher Gensini scores than in groups with a lower Gensini score.¹⁶

C-Reactive Protein (CRP) is one of the acute reactant phase proteins produced by the liver as a marker of systemic inflammation and increases in various types of injury. Previous studies have shown that CRP is not only a sign of inflammation but can play an active role in atherogenesis. Syed SH, *et al.* (2013) conducted a study on the relationship of CRP levels with the severity of stenosis in patients with coronary heart disease. The results showed that there was a significant positive relationship between CRP levels and the severity of stenosis in the group of patients with coronary heart disease.¹⁷ High sensitivity CRP examination techniques have been developed to detect lower serum CRP levels than previous laboratory methods and are known as high sensitive methods CRP (hs-CRP). This technique can be used to assess the risk of heart disease associated with atherosclerotic vascular chronic inflammation.^{17,18}

Stable coronary heart disease occurs with chronic inflammation, therefore the laboratory parameters used in this study were the number of leukocytes, NLR, and CRP as a marker of inflammation in stable coronary heart disease, in which the three parameters affect atherosclerosis.¹⁶ Leukocyte count and NLR can be an important measure of inflammation in stable coronary heart disease because it is cost effective, easily available and can be easily calculated. In addition, CRP is a laboratory parameter that is expected to detect early occurrence of chronic processes in stable coronary heart disease, where laboratory examination is a non-invasive examination. Determination of the stenosis degree used in this study was based on the degree of quantitative coronary stenosis obtained from the results of coronary angiography or cardiac catheterization because it is easier, more done and more applicable.¹⁹ These things encouraged researchers to prove the relationship of leukocyte counts, NLR and CRP levels with stable stenosis in coronary heart disease.

METHODS

A descriptive analytic study with cross-sectional approach was carried out on 35 stable coronary heart disease patients diagnosed by cardiologist at Dr. Kariadi Hospital Semarang. The study

was conducted in March-June 2018. Sample was collected by consecutive sampling after fulfilling the inclusion and exclusion criteria. The inclusion criteria were those aged over 40 years old and willing to take part in the research. Exclusion criteria were respondents experiencing acute coronary syndrome, hematological malignancies, chemotherapy, chronic liver disease, and infections.

The independent variables of this study were the number of leukocytes, NLR, and CRP with a ratio scale. Neutrophil lymphocyte ratio was calculated by dividing between absolute neutrophil counts and absolute lymphocyte counts. The dependent variable was the degree of coronary artery stenosis determined by the Gensini score in the form of the ratio scale. The Gensini score was determined by two cardiologists when performing coronary angiography and then the kappa test was performed. Leukocyte counts and NLR were examined by Sysmex XN hematology analyzer, while CRP was examined by using i-chroma reader.

Data analysis used Spearman test. Significance was stated at $p < 0.05$. Research permits obtained ethical clearance from the Health Research Ethics Commission, Faculty of Medicine, Diponegoro University.

RESULTS

Characteristics of this research subject can be seen in table 1 as follows:

Table 1 shows that the research subjects were dominated by male, namely 21 respondents for male (60%) and 14 respondents (40%) for female. The mean age of respondents was 55.66 years old with a range of ages 43 to 78 years old.

Calculation of the Body Mass Index required data on height and weight. Height in respondents had an average of 160.17 ± 7.05 cm with a minimum height was 150 cm and a maximum of 176 cm. Average respondent's weight was 62.31 ± 9.73 kg with a minimum weight of 45 kg and a maximum of 85 kg. Body mass index was calculated and obtained a mean of 24.10 ± 3.43 kg/m² with a minimum value of 17 kg/m² and a maximum value of 31.2 kg/m².

The results of statistical analysis of leukocyte, NLR and CRP counts on the degree of coronary artery stenosis in stable coronary heart disease are presented in Table 2 below:

Spearman's analysis showed that there was no correlation between the number of leukocytes and CRP with the degree of coronary artery stenosis in stable coronary heart disease. There was a significant positive correlation between NLR and the degree of coronary artery stenosis in stable coronary heart disease.

Table 1 Characteristics of research subjects

Characteristics	N (%)	Mean ± SD	Median (Min- Max)
Sex	35 (100%)	-	-
• Male	21 (60 %)		
• Female	14 (40 %)		
Age (year)	-	55.66 ± 7.37	56 (43-78)
Height (cm)	-	160.17 ± 7.05	160.0 (150-176)
Weight (kg)	-	62.31 ± 9.73	61 (45-85)
Body Mass Index (kg/m ²)	-	24.10 ± 3.43	24.20 (17 – 31.2)
Leukocytes count (/μl)	-	7.54 ± 2.03	7.28 (3.24-13.2)
Absolute neutrophil counts (/μl)	-	4.19 ± 1.32	4.19 (0.76-7.18)
Absolute lymphocyte counts (/μl)	-	2.14 ± 0.60	2.12 (1.19-3.54)
NLR	-	2.10 ± 0.93	1.78 (0.29-4.61)
CRP (mg/L)	-	2.23 ± 1.68	1.5 (0.50-6.54)
Gensini score	-	52.66 ± 42.50	42.5 (0-151)

Table 2 Results of statistical analysis of leukocyte, NLR and CRP counts on the degree of coronary artery stenosis

Variable	Correlation	Significance (p)*
Leukocytes count	0.189	0.277
NLR	0.593	0.000
CRP	0.112	0.521

* The analysis used Spearman test, p < 0.05

DISCUSSION

Table 1 shows that the research subjects were dominated by male, namely 21 respondents for male (60%) and 14 respondents (40%) for female. The average age of respondents was 55.66 years old with range of ages 43 to 78 years old.

This study invited 35 people with stable coronary heart disease who were willing to take part in the study and met the inclusion and exclusion criteria. Research subjects with male sex were more than females subject, namely 21 respondents (60%) compared to 14 respondents (40%). This data shows the incidence of coronary heart disease is more common in men than women. Estrogen hormone protects women from the risk of coronary heart disease. Inflammation is an important element of atherosclerotic pathogenesis and development of coronary heart disease. The estrogen hormone has been shown to increase the expression of superoxide dismutase (SOD) and inhibit the activity of NADPH oxidase thereby reducing oxidative stress. Estrogen hormone has cardioprotection ability.^{20,21}

The average age of respondents was 55.66 years old with a range of ages 43 to 78 years old. This is consistent with the results of Riskesdas (2013) in which the prevalence of coronary heart disease increases with age.³ Increased age is associated

with changes in structure of the coronary arteries, i.e the arterial wall becomes more rigid. Changes in molecular biology in elderly patients also cause arterial endothelial dysfunction.²²

The results of this study showed that there was no significant correlation between the leukocytes counts and CRP with the degree of coronary stenosis. Atherosclerosis is a disorder caused by inflammation. The mechanism of leukocytes in the process of atherosclerosis through the secretion of proteolytic enzymes damage endothelial cells then trigger leukocyte and platelet aggregation processes. This results in microvascular blockages, decreased microvascular perfusion, increased expression of monocyte tissue factor, activation of the coagulation system, increased thrombus formation and electrical instability and the interaction with other atherosclerotic risk factors will increase leukocyte adhesion in the coronary arteries and infarct expansion.¹² Process in stable coronary heart disease occurs minimally in contrast to the conditions that occur in the ACS. This explains why there is no significant correlation between leukocyte count and coronary artery stenosis degree in this study. In a study conducted by Park, *et al* (2004) with multiple regression analysis showed

an increase in the number of leukocytes in CAD and leukocytes as independent risk factors but not statistically significant.²³

C-Reactive Protein is one of the acute reactant phase proteins produced by the liver as a marker of systemic inflammation and increases in various types of injury. Previous studies have shown that CRP is not only a sign of inflammation but can play an active role in atherogenesis.¹⁷ Stable coronary heart disease is known as chronic inflammation and low-grade inflammation. However, stable coronary heart disease also occurs in cell activation, pro-inflammatory cytokines and neutrophils, but the degree of inflammation is not as high as in ACS. This makes CRP levels in patients not statistically significant in line with the study conducted by Johann *et al.* (2002).²⁴

The results of this study reveal that there was a correlation between NLR and degree of stenosis.³ Neutrophil/lymphocyte ratio had been used as a marker of systemic inflammation. NLR was reported to be associated with the severity of heart disease and its clinical output.¹² Sahin, *et al.* (2013) reported that there was a relationship between NLR and the severity of coronary heart disease, where high NLR was a poor prognostic factor in patients with ACS.²⁵ The atherogenesis process is an active process, an inflammatory process with important actions of functional or dysfunctional leukocytes. Neutrophils play an important role in eliminating infarcts or in aggregate formation of platelet leukocytes and in reperfusion injuries in coronary heart disease.¹²

Neutrophil lymphocyte ratio can also be a predictor of mortality in cardiovascular disease.¹⁴ The results of this study are in line with previous studies which showed a relationship between NLR and stenosis severity in stable coronary heart disease based on results of coronary angiography.¹⁵ There were significant differences in NLR count in groups with higher Gensini scores than the group with a lower Gensini score.¹⁶

This study took a stable population of coronary heart disease with comorbid diseases, namely diabetes mellitus, hypertension, dyslipidemia, and smokers who were not analyzed. These diseases in patients with coronary heart disease occur separately or join one another. The NLR parameter can be used as a marker to evaluate patients with diabetes mellitus, hypertension, dyslipidemia and smokers who are likely to later suffer from stable coronary heart disease.

CONCLUSION

There is no relationship between the leukocytes counts and CRP with the degree of coronary artery

stenosis in stable coronary heart disease. There is a significant positive relationship between NLR and coronary artery stenosis which can be used as a marker for evaluating patients who have a high risk of stable coronary heart disease.

ETHICAL CLEARANCE

This study has been received ethical approval by Ethics Committee of Diponegoro University, Semarang, Indonesia.

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding manuscript

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AUTHOR'S CONTRIBUTION

Edward Kurnia Setiawan L and Purwanto Adhipireno conceived of the presented idea. Edward Kurnia Setiawan L developed the theory and Imam Budiwiyono verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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