

# Relationship between Neutrophil to Lymphocyte Ratio (NLR) and Gensini Score in Patients with

*by Deasy Irawati*

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Original Research

**Relationship between Neutrophil to Lymphocyte Ratio (NLR) and Gensini Score in Patients with Coronary Artery Disease (CAD) at Mataram City General Hospital: A Cross Sectional Study**Ni Putu Emi Januantari<sup>1\*</sup>, Deasy Irawati<sup>1,2</sup>, Basuki Rahmat<sup>1,2,3</sup><sup>1</sup>Faculty of Medicine, University of Mataram, Mataram, Indonesia.<sup>2</sup>Lecturer, Faculty of Medicine, University of Mataram, Mataram, Indonesia.<sup>3</sup>Department of Cardiology and Vascular Medicine, Mataram City General Hospital, Mataram, Indonesia.

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## ABSTRACT

**Background.** Neutrophil to Lymphocyte Ratio (NLR) is a marker of systemic inflammation correlated with the incidence of Coronary Artery Disease (CAD). Neutrophils infiltrate the coronary artery plaque causing acceleration of atherosclerosis. T lymphocyte recruitment is required at the early stages of plaque formation. Previous studies have shown that heightened NLR was correlated with the percentage of stenosis at the site of the multivessel lesion.

**Aims.** This study aimed to determine the relationship between NLR with Gensini score of coronary arteries in CAD patients at Mataram City General Hospital.

**Materials and method.** This was a cross sectional study conducted in Mataram City General Hospital. Fifty-nine patients diagnosed with CAD participated in the study. Blood collection and coronary angiography were performed to assess NLR and Gensini Score. The main variables were NLR and Gensini Score. Gensini score of 1-29 was considered as mild atherosclerosis and Gensini score of 30 or more was considered as severe atherosclerosis. NLR was considered high if the value of NLR were > 2.385 based on previous studies. Spearman test was performed to assess correlation between variables.

**Results.** Approximately 53% subjects were categorized as severe atherosclerosis and 48% as mild atherosclerosis. The median values of Gensini score in the low NLR group were 25.0 and in the high NLR group were 40.0 (p=0,19). The median values of NLR in the mild group of atherosclerosis were 1.78 and in the severe atherosclerotic group were 2.15 (p=0,59). There was no significant difference in NLR between severe atherosclerosis and mild atherosclerosis groups. NLR was not associated with Gensini score (p=0,32).

**Conclusions.** There was no significant relationship between Neutrophil to Lymphocyte Ratio with Gensini score of coronary arteries in CAD patients at Mataram City General Hospital.

## Highlights:

1. It discusses the relationship between NLR and the severity of coronary atherosclerosis.
2. There is significant difference from the previous NLR research.

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## Introduction

Atherosclerosis is a major cause of mortality and morbidity in developing countries. Previous studies have shown that inflammation plays a crucial role in the pathogenesis of atherosclerosis, which involves immune cells, lipids, thrombosis, and blood vessel wall components [1]. Inflammation plays a role in every process of atherosclerotic plaque formation from initiation to development, and to thrombotic complications. Leukocytes play a significant role in atherosclerosis and its influence on the risk of cardiovascular events [2]. As many as 45% of deaths from cardiovascular disease are caused by CAD. Coronary Artery Disease causes 7.2 million deaths per year which is 12% of all deaths worldwide. In addition, the World Health Organization (WHO) stated that 60% of the global burden of CAD occurs [11]

Neutrophil to Lymphocyte Ratio is a marker of systemic inflammation associated with CAD. Neutrophils will infiltrate the plaque and accelerate the process of atherosclerosis. Elevated inflammatory markers indicate vascular damage. An increase in NLR indicates the percentage rate of stenosis at the site of multivessel lesions, coronary artery calcification, and intracoronary thrombus.[6,7]

[12] An increase in the number of neutrophils (neutrophilia) refers chronic and adaptive response to myocardial ischemia. Further, lymphocytes play a role in modulating the inflammatory response in the process of atherosclerosis [8]. Recruitment of T lymphocytes especially CD4+ cells, dendritic cells,

and CD8+ T cells occurs at the atherosclerosis prelesional stage. T cells will migrate into atherosclerotic lesions causing lymphocyte cell proliferation which increases the inflammatory response and activates macrophages in the process of atherosclerosis [9]. The lymphocyte count significantly decreased which led to an increase in NLR in CAD patients.[10]

One of the scores to assess the severity of CAD is the Gensini score. The Gensini score is a scoring system invented by Gofredo Gensini in 1983 to determine the severity of CAD. The basic concept in this system is to multiply the coronary artery occlusion score by the coronary artery location score involved to assess the severity of CAD.[11]

Based on previous studies, NLR values were found to be significantly higher in the chronic total coronary occlusion (CTO) group of CAD patients. CTO is defined as complete coronary artery occlusion with no flow (TIMI 0) and occurs for a minimum duration of 3 months [12]. A study in India, patients with abnormal CAG had a significantly higher NLR than normal CAG patients [10]. Previous studies have shown that the lymphocyte count is significantly lower in CAD patients compared to patients without CAD which causes the NLR value to increase in patients with CAD.[13]

NLR is a cheap and easy-to-obtain marker that is [7] useful for cardiac risk stratification in patients with

CAD. Thus, it can reduce health costs if the severity of CAD patients can be detected with NLR. Therefore, researchers are interested in conducting this study, aiming to determine the relationship between NLR and the severity of coronary atherosclerosis, as measured using the Gensini score in patients undergoing Coronary Angiography (CAG). The research was conducted at Mataram City Hospital, West Nusa Tenggara from October to December 2017.

## Material and Methods

### Methods

#### Research Design

This research is a correlative analytic research with cross sectional design. All respondents underwent NLR and Coronary Angiography (CAG) examinations. Patients with suspected CAD at the Mataram City Hospital who met the inclusion and exclusion criteria underwent a complete blood count to obtain the NLR value. On the next day, the patient who had a complete blood count underwent a CAG examination to identify the location and percent of coronary artery blockage. The CAG examination results were then converted to the Gensini scoring system with the final Gensini score and then a comparison was made between NLR and Gensini Score in patients with CAD.

#### Population and Sample

The study population was <sup>1</sup> patients who had been diagnosed with CAD based on clinical symptoms,

physical examination and supporting examinations such as ECG and echocardiography, and had received standard CAD management therapy and had coronary artery angiography (CAG) performed at the Mataram City Hospital, West Nusa Tenggara. The inclusion criteria in this study were patients who had performed CAG, had a history of Acute Coronary Syndrome (ACS), suffered from stable angina pectoris with a treadmill + and agreed and signed a consent statement as research subjects. Exclusion criteria in this study were patients suffering from acute ACS, suffering from infectious diseases, having a history of malignancy, and currently suffering from autoimmune diseases. The sample in this study was selected using a non-probability sampling technique (consecutive sampling) where the research subjects were selected by including respondents who met the inclusion criteria until all the required samples were fulfilled [14]. This research was approved by the Health Research Ethics Committee at the University of Mataram with the ethical number: 01/UN18.8/ETIK/2018.

#### Neutrophil to Lymphocyte Ratio (NLR)

The independent variable in this study is NLR. The NLR value of the study subjects was obtained from a complete blood count by dividing the neutrophil count and lymphocyte count. NLR is declared high if the NLR value is  $> 2.385$ .<sup>[8]</sup>

### Coronary Angiography (CAG)

The dependent variable in this study is the Gensini score. The Gensini scores of the study subjects were obtained from the CAG results of CAD patients converted to the Gensini scoring system. The Gensini score is considered as mild atherosclerosis if the Gensini score is in the range 1-29, if the Gensini score is  $\geq 30$ , it is considered as severe atherosclerosis [15]. The Gensini score method defines coronary artery lumen narrowing as 1 for stenosis of 1 to 25%, 2 for 26 to 50%, 4 for 51 to 75%, 8 for 76 to 90%, 16 for 91 to 99%, and 32 for total occlusion. The score is then multiplied by the score based on the location of the coronary arteries. For coronary artery location score; 5 points for left main lesion (LM); 2.5 for the proximal segment of the left anterior descending artery (LAD) or left circumflex artery (LCX); 1.5 for the mid LAD and LCX segments; 1 for the distal segments of the LAD and LCX, first diagonal branch, first obtuse marginal branch, right coronary artery (RCA), posterior descending artery, and intermediate artery; and 0.5 for the second branch of the diagonal and the obtuse marginal.[8]

### Risk Factors

This study also analyzed risk factor variables, such as lifestyle (i.e. history of smoking) and history of comorbid diseases (i.e. diabetes mellitus and hypertension). Other variables analyzed in this study were age, gender, interpretation of blood pressure,

body mass index (BMI), diagnosis, leukocyte, neutrophil, and lymphocyte counts. The interpretation of blood pressure in this study uses the American Heart Association classification, which divides the interpretation into normal blood pressure, prehypertension, stage 1 hypertension and stage 2 hypertension [16]. The interpretation of body mass index in this study used the Indonesian Ministry of Health's BMI criteria, with four categories; underweight, normal, overweight, and obese [17]. In this study, the normal category of leukocytes were  $3.54 - 9.06 \times 10^9/L$ , neutrophils were  $1.42 - 6.34 \times 10^9/L$ , and lymphocytes were  $0.71-4.53 \times 10^9/L$ . [18]

### Statistical Analysis

The relationship between characteristics and risk factor variables with the main variable was tested using Chi Square bivariate test analysis. In addition, the analysis of the main variable correlation test, namely the relationship between NLR and Gensini score using Spearman's non-parametric correlation test because the data distribution is not normal after the data normality test is carried out using the Kolmogorov-Smirnof test (sample > 50 people). Data in this study were also analyzed using the Mann Whitney non-parametric comparison test to determine if there were differences in NLR values between the mild atherosclerosis group and the severe atherosclerosis group. As well as the difference in Gensini scores between the high NLR group and the low NLR group.

## Results

### Characteristics of Research Subjects

This study found 59 research subjects met the inclusion and exclusion criteria. Most of the research subjects were male (72.9%) and aged over 55 years (61.0%). As many as 8.6% (11 people) of the study subjects had a non-significant CAD diagnosis, 13.6% (8 people) had a CAD 1 VD diagnosis, 27.1% (16 people) had a CAD 2 VD diagnosis, and 40.7% (24 people) had a diagnosis of CAD 3 VD. As many as 23.7% (14 people) of the research subjects were included in the obese group. As many as 54.2% (32 people) of the study subjects suffered from

hypertension. As many as 37.3% (22 people) of the research subjects suffered from diabetes mellitus.

Considering that gender is a risk factor for CAD, in this study data were also analyzed based on gender. Based on the results of data analysis, it was found that there was a significant relationship between gender and smoking history ( $p < 0.001$ ). Gender was not significantly related to age, BMI, hypertension status and diabetes status.

Table 1. Distribution of research subjects based on the Gensini score

| Parameter                       | Mild atherosclerosis n (%) | Severe atherosclerosis n (%) | Total n (%) | P value |
|---------------------------------|----------------------------|------------------------------|-------------|---------|
| Age (n=59)                      |                            |                              |             | 0,56    |
| <55 years                       | 12(42,9)                   | 11(35,5)                     | 23(39,0)    |         |
| ≥55 years                       | 16(57,1)                   | 20(64,5)                     | 36(61,0)    |         |
| Sex (n=59)                      |                            |                              |             | 0,81    |
| Male                            | 20(71,4)                   | 23(74,2)                     | 43(72,9)    |         |
| Female                          | 8(28,6)                    | 8(25,8)                      | 16(27,1)    |         |
| BMI (n=59)                      |                            |                              |             | 0,35    |
| Underweight                     | 1(3,6)                     | 1(3,2)                       | 2(3,4)      |         |
| Normal                          | 14(50,0)                   | 22(71,0)                     | 36(61,0)    |         |
| Overweight                      | 4(14,3)                    | 3(9,7)                       | 7(11,9)     |         |
| Obese                           | 9(32,1)                    | 5(16,1)                      | 14(23,7)    |         |
| Hypertension Status (n=59)      |                            |                              |             | 0,41    |
| No                              | 12(42,9)                   | 15(48,4)                     | 27(45,8)    |         |
| Yes                             | 16(57,1)                   | 16(51,6)                     | 32(54,2)    |         |
| Diabetes Mellitus Status (n=59) |                            |                              |             | 0,18    |
| No                              | 20(71,4)                   | 17(54,8)                     | 37(62,7)    |         |
| Yes                             | 8(28,6)                    | 14(45,2)                     | 22(37,3)    |         |
| History of Smoking (n=59)       |                            |                              |             | 0,21    |
| Non smoker                      | 18(64,3)                   | 15(48,4)                     | 33(55,9)    |         |
| Smoker                          | 10(35,7)                   | 16(51,6)                     | 26(44,1)    |         |
| Leukocyte Count (n=59)          |                            |                              |             | 0,94    |
| Below normal                    | 0                          | 0                            | 0           |         |
| Normal                          | 16(57,1)                   | 18(58,1)                     | 34(57,6)    |         |
| Above normal                    | 12(42,9)                   | 13(41,9)                     | 25(42,4)    |         |



|                                |          |          |          |             |
|--------------------------------|----------|----------|----------|-------------|
| <b>Neutrophil count (n=59)</b> |          |          |          | <b>0,13</b> |
| Below normal                   | 0        | 0        | 0        |             |
| Normal                         | 25(89,3) | 23(74,2) | 48(81,4) |             |
| Above normal                   | 3(10,7)  | 8(25,8)  | 11(18,6) |             |
| <b>Lymphocyte count (n=59)</b> |          |          |          | <b>1,00</b> |
| Below normal                   | 0        | 0        | 0        |             |
| Normal                         | 27(96,4) | 30(96,8) | 57(96,6) |             |
| Above normal                   | 1(3,6)   | 1(3,2)   | 2(3,4)   |             |

\*Statistical analysis with Chi Square test

*Atherosclerosis Severity in Research Subjects*

<sup>1</sup>Based on the Gensini score, it was found that 47.5% (28 people) of the study subjects had mild atherosclerosis and 52.5% (31 people) had severe atherosclerosis (Table 1). In the severe atherosclerosis group, 64.5% (20 people) were over 55 years old, 74.2% (23 people) were male subjects, 16.1% (5 people) were included in the obese group, 51.6% (16 people) suffered from hypertension,

45.2% (14 people) had diabetes mellitus, and 51.6% (16 people) were smokers. In this study, there was <sup>3</sup>no significant relationship between the severity of CAD and the above risk factors. In addition, there was also <sup>3</sup>no significant relationship between the severity of CAD and the number of leukocytes, neutrophils, and lymphocytes.

Table 2. Distribution of research subjects based on NLR

| Parameter                              | Low NLR<br>n (%) | High NLR<br>n (%) | Total<br>n (%) | P<br>value |
|--|------------------|-------------------|----------------|------------|
| <b>Age (n=59)</b>                      |                  |                   |                |            |
| <55 years                              | 13(34,2)         | 10(47,6)          | 23(39,0)       | 0,31       |
| <sup>16</sup> ≥55 years                | 25(65,8)         | 11(52,4)          | 36(61,0)       |            |
| <b>Sex (n=59)</b>                      |                  |                   |                |            |
| Male                                   | 27(71,1)         | 16(76,2)          | 43(72,9)       | 0,67       |
| Female                                 | 11(28,9)         | 5(23,8)           | 16(27,1)       |            |
| <b>Diagnosis (n=59)</b>                |                  |                   |                |            |
| Non-significant CAD                    | 8(21,1)          | 3(14,3)           | 11(18,6)       | 0,33       |
| CAD 1 VD                               | 6(15,8)          | 2(9,5)            | 8(13,6)        |            |
| CAD 2 VD                               | 12(31,6)         | 4(19,1)           | 16(27,1)       |            |
| CAD 3 VD                               | 12(31,6)         | 12(57,1)          | 24(40,7)       |            |
| <b>BMI (n=59)</b>                      |                  |                   |                |            |
| Underweight                            | 2(5,3)           | 0(0)              | 2(3,4)         | 0,35       |
| Normal                                 | 20(52,6)         | 16(76,2)          | 36(61,0)       |            |
| Overweight                             | 5(13,2)          | 2(9,5)            | 7(11,9)        |            |
| Obese                                  | 11(28,9)         | 3(14,3)           | 14(23,7)       |            |
| <b>Hypertension Status (n=59)</b>      |                  |                   |                |            |
| No                                     | 19(50,0)         | 8 (38,1)          | 27(45,8)       | 0,38       |
| Yes                                    | 19(50,0)         | 13(61,9)          | 32(54,2)       |            |
| <b>Diabetes Mellitus Status (n=59)</b> |                  |                   |                |            |
|  |                  |                   |                | 0,30       |

|                           |          |          |          |      |
|---------------------------|----------|----------|----------|------|
| No                        | 22(57,9) | 15(71,4) | 37(62,7) |      |
| Yes                       | 16(42,1) | 6(28,6)  | 22(37,3) |      |
| History of Smoking (n=59) |          |          |          | 0,21 |
| Non smoker                | 19(50,0) | 14(66,7) | 33(55,9) |      |
| Smoker                    | 19(50,0) | 7(33,3)  | 26(44,1) |      |
| Gensini score (n=59)      |          |          |          | 0,28 |
| Mild atherosclerosis      | 20(33,9) | 8(13,5)  | 28(47,5) |      |
| Severe atherosclerosis    | 18(30,5) | 13(22,1) | 31(52,5) |      |

\*Statistical analysis with Chi Square test

#### Level of NLR Value in Research Subjects

The characteristics of the NLR values of the research subjects are shown in Table 2. Based on the NLR values, it was found that 64.4% (38 people) of the research subjects had low NLR values and 35.6% (21 people) of the research subjects had high NLR values. In this study, 22.1% (13 people) of research subjects with high NLR had severe atherosclerosis. As many as 52.4% (11 people) of the high NLR subject group were over 55 years old. Based on the results of data analysis, there was no significant relationship between the NLR value and the above risk factors.

#### Relationship between NLR and Gensini score

The results of the Mann Whitney comparative test analysis for NLR scores between the high-score Gensini group and the low-score Gensini group showed no significant differences ( $p=0.59$ ). This result is supported by the results of the Mann Whitney test for the Gensini score between the high NLR group and the low NLR group which also showed no significant difference ( $p=0.19$ ). For Spearman's correlative test results between NLR

and Gensini score, there is no significant relationship between NLR and Gensini score (Figure 1).

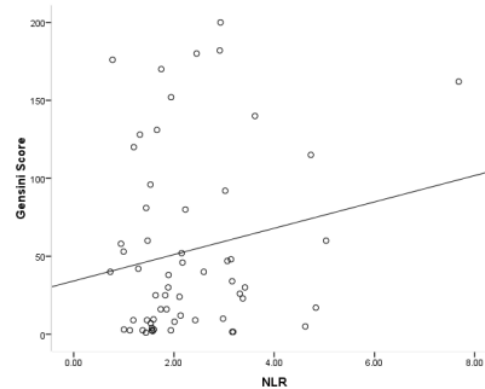


Figure 1. Correlation between NLR and Gensini score

#### Discussion

This study aims to examine the relationship between the Neutrophil to Lymphocyte Ratio (NLR) and the Gensini score of the coronary arteries in Coronary Artery Disease (CAD) patients. In this study, 47.5% (28 people) of subjects were classified as mild atherosclerosis and 52.5% (31 people) were classified as severe atherosclerosis. As many as 22.1% (13 people) of subjects with severe atherosclerosis had high NLR values.



Previous research explained that an increase in NLR and a decrease in the number of lymphocytes correlated significantly with the severity of CAD as assessed using the Gensini score [10]. Neutrophil to Lymphocyte Ratio (NLR) is a simple parameter to assess a subject's systemic inflammatory status. This has proven useful in stratifying mortality in major cardiovascular disease [19]. NLR is obtained by dividing the number of neutrophils by the number of lymphocytes. NLR is prognostic of acute coronary syndrome and is a more reliable predictor than other predictors based on leukocyte data. NLR is an inflammatory marker that can predict death, myocardial infarction, and coronary artery disease. A high NLR is prognostic of atherosclerotic development.[20]

Neutrophils play a role in every process in acute coronary events from initiation to plaque rupture, by releasing both prothrombotic and proactive substances, which cause endothelial damage and platelet aggregation. The effect of increased levels of neutrophils (neutrophilia) on CAD can be explained by the secretion of various inflammatory mediators such as elastase, myeloperoxidase and oxygen free radicals which cause tissue damage.[7,21]

A low lymphocyte count is also associated with a poorer prognosis in patients with CAD and unstable angina, because several subsets of lymphocytes have been shown to play a role in inhibiting atherosclerosis. This subset of lymphocytes is a

subtype of T-lymphocytes namely regulatory T cells (including CD4+CD25+ T cells) which have been found to play an inhibitory role in atherosclerosis.

Regulatory T cells cause upregulation of the inhibitory cytokine interleukin 10 (IL-10) and transforming growth factor (TGF- $\beta$ ), leading to a reduction in atherosclerotic plaque accumulation and a potential transition from a T helper 1 (Th1) to a T helper 2 (Th 2) response, thereby supporting B cell atheroprotective responses and inhibiting proatherogenic Th immune responses [7,21]. Possible causes of lymphopenia are decreased production induced by increased steroid levels due to stress and increased apoptosis induced by increased inflammation [21]. An increase in neutrophils accompanied by a decrease in lymphocytes results in an increased NLR in the CAD and severe atherosclerosis groups.[13,15]

In the study of Kaya et al (2014), it was found that the NLR was significantly higher in the severe atherosclerosis group (Gensini score  $\geq 30$ ) compared to mild atherosclerosis (Gensini score = 1-29) and the control group [15]. Another researcher, Ates et al (2016), also published that NLR levels were found to be higher in patients with Critical Stenosis than patients without Critical Stenosis [22]. Research by Allichandi & Khilari (2016) also showed that the number of lymphocytes decreased significantly in patients with CAD and the ratio of neutrophils and lymphocytes increased in patients with CAD. In

addition, this study also reported a significant correlation between the number of lymphocytes and NLR with the severity of CAD through the assessment of the Gensini score [10]. The NLR was significantly higher in the CTO group of CAD patients [12]. CTO is defined as total coronary artery occlusion with no flow (TIMI 0) and occurs for a duration of at least 3 months. In a study by Selcuk et al (2012) in Turkey, it was also found that there was a decrease in the number of lymphocytes and an increase in NLR in patients with significant CAD compared to patients without CAD.[12,13]

The main finding in this study is that there is no significant relationship between NLR and Gensini score. This is contrary to the results of previous studies which showed a significant relationship between NLR and Gensini score of coronary artery in CAD patients [8,12,15]. A study by Uysal et al (2016) explained that NLR is a predictor of severe atherosclerosis and can be used as a predictive factor and risk identification for heart disease in CAD patients. Uysal et al (2016) also used a control group or a group with no CAD diagnosis. Whereas, this study only used a group of research subjects diagnosed with CAD. Previous studies also using research subjects with coronary artery blockage of at least 50% in at least one coronary artery [23,24]. Whereas in this study, all research subjects were CAD patients with various degrees of stenosis. The

gaps above can contribute to differences in the results of this study with previous studies.

Another factor that can influence the difference in the results of this study with previous study is the subject medical history. In a previous study, it was found that nebivolol could reduce NLR values more than metoprolol in patients with hypertension. In this study, the authors did not consider the patient's medication history so that these factors could affect the NLR value in patients who had previously taken medication [5]. This is supported by the research of Zhang, et al (2014) who performed NLR examinations of patients before starting any treatment [6]. It can be a factor that influences the difference in the results of this study with previous studies.

Other factors that may influence the results are exercise and hydration levels [25]. In this study, the authors did not consider sports history and did not measure the degree of hydration in the research subjects. This can contribute to differences in the results of this study with previous studies.

The study also found a significant relationship ( $p<0.05$ ) between smoking and gender. This is supported by a prevalence study by WHO which shows that there are more male smokers in Indonesia than female smokers, it is about 64.9% of male smokers [26]. Previous studies also found that there are significant sex differences between men

and women in CAD. Epidemiological studies showed the incidence of coronary artery disease is greater in males than females. This is caused by a woman's physiological factors namely estrogen. Estrogen is considered beneficial because it has effects on atherosclerotic plaques, vasodilation, blood pressure, and has antioxidant and anti-inflammatory effects.<sup>[27]</sup>

This study is not free from various limitations. The limitation of this study includes confounding factors that cannot be assessed, such as medical history, degree of hydration and physical activity (exercise). The second limitation is that this study uses a cross-sectional method so that it cannot show a causal relationship between variables. In addition, this study was only conducted at one <sup>1</sup> hospital (single center based study) so that the sample used was not representative of CAD patients in the general population.

### Conclusion

One out of two CAD patients at the Mataram City General Hospital is classified as having severe atherosclerosis. Based on NLR values, one in three CAD patients has high NLR. In the results of this study showed there was no significant relationship <sup>2</sup> between NLR and Gensini coronary artery scores in CAD patients at Mataram City Hospital.

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