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## Association of Neutrophil Lymphocyte Ratio (NLR) with Global Registry of Acute Coronary Events (GRACE) Scores in Acute Coronary Syndrome

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**Abstract. Background.** Inflammation plays an important role in the initiation of atherosclerosis from the beginning of plaque to rupture cause Acute Coronary Syndrome (ACS). Neutrophil Lymphocyte Ratio (NLR) indicator of systemic inflammation in ACS. Risk stratification was needed for assessment and selection of initial invasive strategies and find the best strategy in ACS. The Global Registry of Acute Coronary Events (GRACE) scores recommended risk stratification of ACS. Aims of the study to determine the association and cut-off value NLR with risk stratification GRACE score. **Method.** This study is analytical with a cross-sectional retrospective design. Data were analyzed after distribution test, then mean difference and correlation test was using the SPSS program where  $p < 0.05$  was considered statistically significant. **Results.** This study showed significantly higher NLR value in the high risk stratification and intermediate-risk compared to low risk stratification ( $7.9 \pm 2.7$  vs  $3.6 \pm 1.7$ ;  $p=0.001$ ) ( $5.2 \pm 2.3$  vs  $3.6 \pm 1.7$ ;  $p=0.018$ ). Significant correlation between NLR with GRACE scores ( $r=0.570$ ;  $p<0.001$ ). Significant AUC values were obtained (0.782,  $p < 0.001$ , IK95% 0.674-0.89), and cut-off values NLR 4 with sensitivity (78.8%) and specificity (70.3%) on the GRACE score. **Conclusion.** The significant association between NLR with GRACE risk score in ACS.

**Keyword.** Neutrophil Lymphocyte Ratio, GRACE score, Acute Coronary Syndrome

**Abstrak. Latar belakang.** Inflamasi berperan penting dalam inisiasi terjadinya aterosklerosis mulai dari awal perkembangan plak sampai terjadinya ruptur hingga menyebabkan Sindrom Koroner Akut (SKA). Neutrophil Lymphocyte Ratio (NLR) merupakan indikator inflamasi sistemik pada SKA. Stratifikasi risiko diperlukan untuk penilaian dan pemilihan strategi invasif awal sehingga menemukan strategi terbaik pada SKA. Global Registry of Acute Coronary Events (GRACE) skor merupakan skoring risiko yang direkomendasikan dalam stratifikasi risiko SKA. Penelitian ini bertujuan mengetahui hubungan dan nilai potong NLR dengan stratifikasi GRACE skor pada SKA. **Metode.** Penelitian retrospektif cross sectional bersifat analitik komparatif. Data dianalisis setelah uji distribusi, kemudian dilakukan uji beda mean dan uji korelasi menggunakan program SPSS di mana  $p < 0,05$  dianggap signifikan secara statistik. **Hasil.** Pada penelitian terdapat Nilai NLR signifikan lebih tinggi pada stratifikasi high risk dan stratifikasi intermediate risk dibandingkan stratifikasi low risk ( $7.9 \pm 2.7$  vs  $3.6 \pm 1.7$ ;  $p=0.001$ ) ( $5.2 \pm 2.3$  vs  $3.6 \pm 1.7$ ;  $p=0.018$ ). Korelasi yang signifikan antara nilai NLR dengan nilai GRACE skor ( $r= 0.570$ ,  $p < 0.001$ ). Didapatkan nilai AUC signifikan (0.782,  $p < 0.001$ , IK95% 0.674-0.89), dan nilai titik potong NLR 4 dengan sensitivitas (78.8%) dan spesifitas (70.3%) terhadap GRACE skor. **Kesimpulan.** Hubungan yang signifikan antara nilai NLR dengan stratifikasi GRACE skor pada SKA.

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**Kata Kunci.** *Neutrophil Lymphocyte Ratio, GRACE Skor, Sindrom Koroner Akut*

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## 1 Introduction

Cardiovascular disease is the major cause of mortality globally. Data the American Heart Association (AHA), the mortality rate due to cardiovascular disease reach 17.8 million in 2017 [1]. Cardiovascular disease mortality rates will increase reach 24.2 million the year 2030. Data the United States more than 1 million people have Acute Coronary Syndrome (ACS) showed 0.4 million people die each year due to ACS [2]. In Indonesia, according to basic health research data in 2018, showed that 15 out of 1,000 Indonesians suffer from coronary heart disease[3].

Inflammation plays an important role in the initiation of atherosclerosis from the beginning of plaque to the occurrence of rupture which causes ACS [2]. Neutrophils as a marker of inflammation and lymphocytes as a marker of regulatory pathways. Neutrophil Lymphocyte Ratio (NLR) as an indicator of systemic inflammation in ACS, integrating both of as predictors mortality of ACS[4].

Risk stratification is needed for the assessment and selection of initial invasive strategies and find the best strategy for achieving and maintaining myocardial reperfusion [5]. Recent guideline recommendation the Global Registry of Acute Coronary Events (GRACE) score for risk stratification and prognosis of patients ACS [6]. The American College of Cardiology Foundation / American Heart Association (ACCF/AHA) guidelines recommends an invasive strategy for patients with high risk GRACE scores (>140) and conservative approaches for patients with low risk GRACE score (<140) [7]. The study of Shuvy et al. [8] found high risk GRACE score (>140) have higher mortality rate than low risk GRACE score (<140) in ACS.

Some of studies show that the combination of GRACE scores with other clinical and laboratory parameters can increase predictive value of ACS [8]. The study of Oncel et al. [9] found that NLR value (>6.48) correlation with high risk GRACE score and NLR value (>2.65) correlation with low risk GRACE score. Aims of the study to determine the association and cut-off value NLR with risk stratification GRACE score in ACS.

## 2 Method

This study is analytical with a cross-sectional retrospective design. The study was conducted at H. Adam Malik General Hospital in January 2020. Secondary data from medical records used as

subjects of study, medical record data from January to December 2019. A total of 70 subjects of study who met the inclusion and exclusion criteria using the consecutive sampling method, consist of stratification low risk (< 109), intermediate-risk (109-140) and high risk (>140).

Analysis and presentation of data using statistical analysis with Stastical Product and Service Solution (SPSS) 22.0 for windows software. Univariate analysis to determine the demographic characteristics of this study. Data were analyzed after distribution test, the results of the study were statistically analyzed using One way annova test if the data were normally distributed, and if the data were not normally distributed the Kruskal wallis test was used. The bivariate analysis uses the Pearson correlation test when it is normally distributed and the Spearman correlation test when it is not normally distributed. ROC analysis was used to determinate of cut-off value. P-value <0.05 was considered statistically significant.

### 3 Results

There were in total 70 medical records of ACS patients that obtained in this study. The characteristics subject of study such as age, gender, blood pressure, BMI, risk factors of cardiovascular and biochemical results are presented in table 1. The majority of subjects were male 65 (92.9%), the mean age of the subjects was  $55.5 \pm 10.8$  years.

Table 1. Demographic and Biochemical Characteristics of Subjects in GRACE Risk Score Groups

Variable	Total (N= 70) X±SD	Low Risk (N=37) X±SD	Intermediate Risk (N=23) X±SD	High Risk (N=10) X±SD	P-value
Age, years	55.5 ± 10.8	49.4 ± 9.3 <sup>a,b</sup>	60.9 ± 7.1	65.4 ± 9.6	0.001 <sup>x**</sup>
Male, n (%)	65 (92.9)	35 (94.6)	20 (87)	10 (100)	
SBP, mmHg	126.7± 26.8	131.9 ± 24	124.8 ±25	112 ± 36	0.112 <sup>y</sup>
BMI, kg/m <sup>2</sup>	26.7 ± 3.9	26.9 ± 3.8	26.1 ± 4.3	27.8 ±3.4	0.475 <sup>y</sup>
Hipertensi, n (%)	42 (60)	19 (51.4)	15 (65.2)	8 (80)	0.567 <sup>z</sup>
DM, n (%)	17 (24.3)	8 (21.6)	7 (30.4)	2 (20)	
Dyslipidemia, n (%)	22 (31.4)	13 (35.1)	7 (30.4)	2 (20)	
Smoke, n (%)	58(82.9)	35 (94.6)	18 (78.3)	5 (50)	0.045 <sup>z*</sup>
Leukosit, cells/mL	11743 ± 3443	12411 ± 3194	11041 ± 3676	10885 ± 3623	0.229 <sup>x</sup>
Neutrophil absolute	8.5 ± 2.97	8.5 ±3.2	7.9 ± 2.7	9.5 ± 2.4	0.377 <sup>x</sup>
Lymphocyte absolute	2.1 ± 0.8	2.5 ± 0.7 <sup>a,b</sup>	1.7 ± 0.6	1.3 ± 0.4	0.001 <sup>x**</sup>
NLR	4.7 ± 2.5	3.64±1.7 <sup>a,b</sup>	5.23 ± 2.33 <sup>b</sup>	7.9 ± 2.7	0.001 <sup>x**</sup>
CKMB	137.5 ± 156	136.5 ± 171	145.6 ± 143	122.6 ± 142	0.41 <sup>y</sup>
Troponin I, ng/dL	9.1 ± 16	8.1 ± 16.3	9.6 ± 18.23	12.04 ± 8.7	0.078 <sup>y</sup>
Glucose random, mg/dl	130 ± 62.3	129.4 ± 39	143.3 ± 94.8	105.3 ± 23.4	0.139 <sup>y</sup>
Creatinine, mg/dl	1.4 ± 1.3	0.9 ± 0.49	1.03 ± 0.34	3.4 ± 2.54 <sup>a,c</sup>	0.001 <sup>y**</sup>
Killip I, n (%)	28 (40)	22 (59.5)	6 (26.1)	-	
II	35 (50)	15 (40.5)	16 (69.6)	4 (40)	
III	3(4.3)	-	1 (4.3)	2 (20)	
IV	4(5.7)	-	-	4 (40)	

x One Way Anova Test

y Kruskal-Wallis Test

z Kolmogorov Smirnov Z Test

<sup>a</sup> P < 0.05 Significant compared with *Intermediate Risk*

<sup>b</sup> P < 0.05 Significant compared with *High Risk*

<sup>c</sup> P < 0.05 Significant compared with *Low Risk*

\* P< 0.05, \*\*P < 0.001

SBP : Systolic Blood Pressure; BMI : Body Mass Index; DM : Diabetes Mellitus; NLR : Neutrophyl Lymphocyte Ratio; CKMB : Creatinine Kinase Myocardial Band

There was significant difference between age with GRACE risk score ( $p < 0.001$ ). Significantly most of older have high risk GRACE score ( $p = 0.001$ ). There was statistically significant association between having a smoke with GRACE risk score ( $p = 0.045$ ).

There was significant difference between absolute lymphocyte count with GRACE risk score ( $p < 0.001$ ), it significantly lower absolute lymphocyte count in high risk compared intermediate-risk and low risk stratification ( $p = 0.001$ ;  $p = 0.001$ ). There was significant difference between NLR values with GRACE risk score ( $p < 0.001$ ). NLR values were significantly higher in high risk and intermediate-risk compared low risk stratification ( $7.9 \pm 2.7$  vs  $3.6 \pm 1.7$ ;  $p = 0.001$ ) ( $5.2 \pm 2.3$  vs  $3.6 \pm 1.7$ ;  $p = 0.018$ ). There was statistically significant difference between creatinine serum with the GRACE risk score ( $p < 0.001$ ). It was found significantly increase creatinine serum in high risk compared intermediate-risk and low risk stratification ( $p = 0.001$ ;  $p = 0.001$ ). Statistically significant association between Killip class with the GRACE risk score ( $p = 0.027$ ).

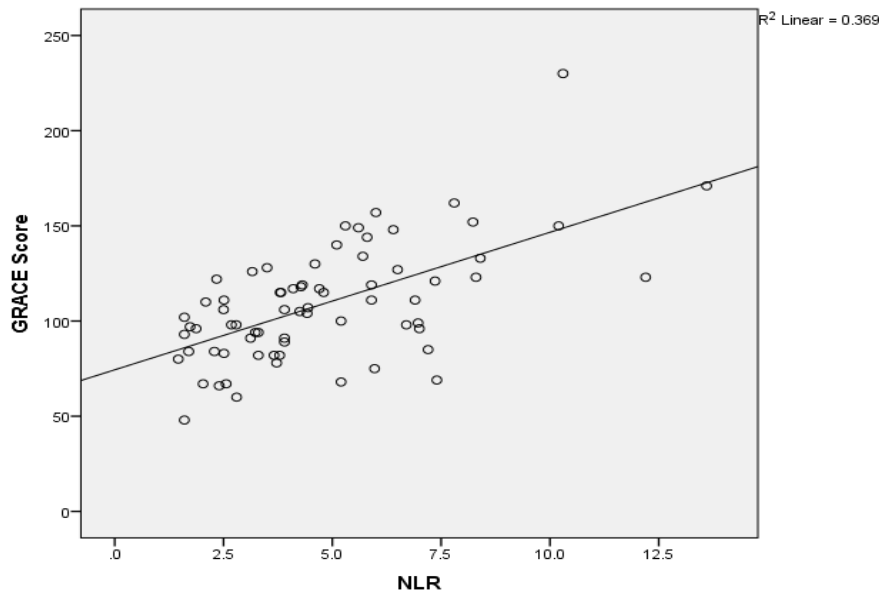


Figure 1. Scatter plot correlation between NLR and GRACE score

In this study, the Spearman correlation test was conducted to find the correlation between NLR and GRACE score. The result show that there is significant correlation between NLR values and GRACE scores ( $r = 0.570$ ;  $p < 0.001$ ) (see Figure 1).

In this study, ROC analysis was used to determinate of cut-off NLR value with GRACE risk score. It was found significant that the AUC value and cut-off value NLR was determined based on the most optimal sensitivity and specificity values in Figure 2.

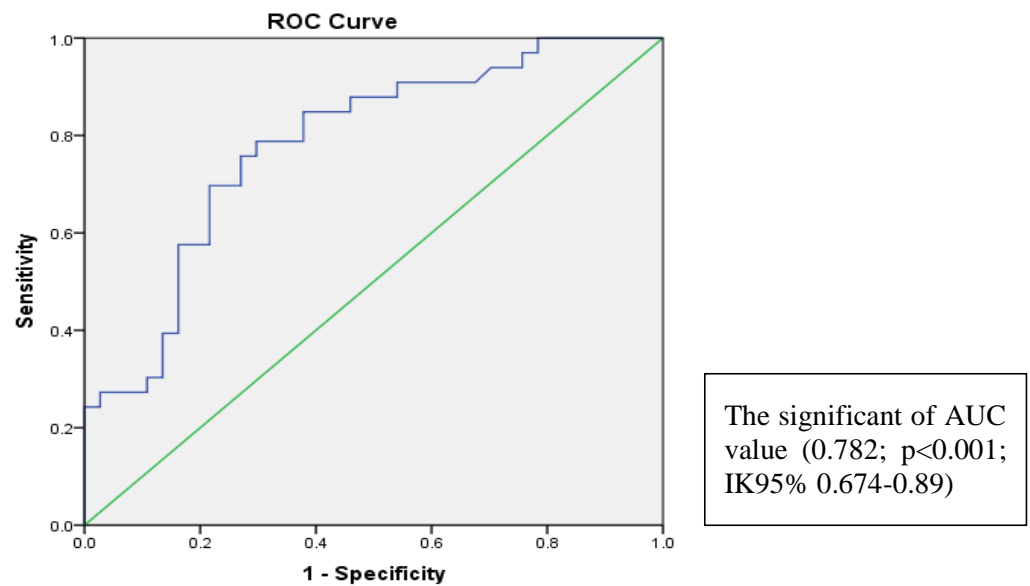


Figure 2. ROC curves for NLR and GRACE risk scores (Cut-off value NLR = 4, sensitivity 78.8%; specificity 70.3%)

#### 4 Discussion

In this study, the majority of subjects were male 65 (92.9%) with mean age of the subjects was  $55.5 \pm 10.8$  years. Similar to the results of this study, Oncel, et.al [9] found that the prevalence of ACS was higher in male (80.2%). The same results of study Firdous [10] have found comparison prevalence of ACS between male and female 3: 2. Endogenous estrogen hormone in females who have protective effect on endothelial blood vessel [11]. Similar results of this study, Adam et.al [12] found mean age of ACS is 55.4 years.

This study showed statistically significant association between having a smoke with the GRACE risk score ( $p=0.045$ ). Similar to the results of this study, Acet et.al [13] found having a smoke as risk factor association significantly with the GRACE risk scores ( $p < 0.001$ ). Smoking can cause inflammation of blood vessels which results in narrowing of the arteries, reducing blood flow and oxygen supply to organs including the heart which can cause myocardial infarction. Smoking causes endothelial injury and dysfunction of the coronary and peripheral arteries, a chronic inflammatory state that contributes to the atherogenic disease process and increases inflammation biomarkers, as a strong predictor of cardiovascular events[14].

Inflammation plays an important role in the initiation and progression of the atherosclerotic process. Inflammation is also known to play a role in all phases of ACS, which will affect the formation and rupture of atherosclerotic plaque [9]. Neutrophils play a role in the formation of atherosclerotic plaques in ACS. The process of forming atheroma plaques in the tunica intima lining of arteries. Endothelial dysfunction occurs due to exposure to irritative stimulus stimuli such as hyperlipidemia, large shear stress and proinflammatory cytokines [15]. In this study, there was no significant difference between absolute neutrophils compared to GRACE risk scores ( $p =$

0.377). Acet et.al found no significant difference between absolute neutrophil with GRACE risk scores ( $p = 0.307$ ) [13]. Neutrophils play a role in acute tissue damage by removing inflammatory mediators. Neutrophils release arachidonic acid and superoxide radicals by proteolytic enzymes, which make the formed plaque more fragile [16]. Plaque destabilization causes rupture of atherosclerosis and injury blood vessel endothelium. Neutrophils adhesion to endothelium capillary so that prevent reperfusion of ischemia capillaries[17].

Lymphocytes have been shown to modulate immunological responses at all stages of the atherosclerotic process. The systemic inflammatory response is characterized by a low lymphocyte count [13][18]. Lymphocytes regulate the inflammatory response and play an antiatherosclerotic role where regulatory T cells have an inhibitory effect of atherosclerosis. Low lymphocyte counts serve as an early marker of physiological stress and systemic inflammation secondary to myocardial ischemia mediated by cortisol release. Increased cortisol levels result in decreased lymphocytes [19]. In this study showed significant difference between absolute lymphocyte count with GRACE risk score ( $p < 0.001$ ), it was found significantly lower absolute lymphocyte count in high risk compared intermediate-risk and low risk stratification ( $1.3 \pm 0.4$  vs  $1.7 \pm 0.6$ ;  $p=0.001$ ) ( $1.3 \pm 0.4$  vs  $2.5 \pm 0.7$ ;  $p=0.001$ ). Acet et al. [13] showed significant difference between absolute lymphocytes compared with GRACE risk score ( $p=0.001$ ).

The combination of neutrophil and lymphocyte parameters have better prognostic value than other parameters. Neutrophils as inflammatory markers and lymphocytes as regulator markers. NLR have a role indicator of systemic inflammation in ACS, integrating both as predictors and mortality of ACS [4][20]. In this study, showed significant difference between NLR values with GRACE risk score ( $p < 0.001$ ). Statistically significant NLR values higher in high risk compared intermediate-risk and low risk stratification ( $p=0.001$ ;  $p=0.018$ ). Oncel et al. [9] showed that the increase in NLR values had positive correlation with GRACE risk score ( $p < 0.001$ ). Acet et.al. [13] expressed the similar results, where there was significant increase NLR values proportional to the GRACE risk score ( $p = 0.008$ ).

In this study, the Spearman correlation test was showed significant correlation between NLR values with GRACE risk scores ( $r=0.570$ ;  $p<0.001$ ). It was found significant that the AUC value (0.782;  $p<0.001$ ; IK95%; 0.674-0.89), and cut-off value NLR 4 was determined based on the most optimal sensitivity (78.8%) and specificity (70.3%) values. Similar to the results of this study Acet et al. [13] showed statistical significant correlations between NLR values and with GRACE risk scores ( $r=0.172$ ;  $p<0.001$ ). The study of Chen et al. [4] found that NLR value ( $> 4$ ) as a strong predictor of myocardial dysfunction in ACS. The higher of NLR ( $> 6.5$ ) were associated with increased 30-day and 5-year mortality [4]. Expressed the similar results, found that NLR values ( $>5$ ) correlated significantly higher ACS mortality in hospital care (OR 6.39;  $p<0.001$ ) [21]. Budzianowski et al. [20] found that NLR ( $> 4.9$ ) have sensitivity (70%) and a

specificity (65%) in predicting hospital mortality. Darmawan et al. [11] found cut-off value NLR 3.55 to increase MACE in patients ACS.

The limitations of the study, this was a retrospective and single-center study that included a relatively small number of patients. This study only one-time measurement of admission full blood count and calculation of NLR were included in the analysis.

## 5 Conclusion

The GRACE risk score is routinely used for the stratification of patient ACS. This study showed that significant association between NLR with GRACE risk score in ACS. NLR value may provide additional prognostic value in ACS. These significant findings of our analysis can guide for further clinical practice. However, these findings must be confirmed in study with a larger number of patients.

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