



## Association Between Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio with Global Registry of Acute Coronary Events Risk Score in Acute Myocardial Infarction

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### ABSTRACT

**Background.** The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are novel inflammatory markers in cardiovascular diseases. Global Registry of Acute Coronary Events (GRACE) risk score is a scoring system to predict the risk of mortality in acute coronary syndrome. **Objective.** To investigate the association between NLR and PLR with GRACE risk score in acute myocardial infarction (AMI) patients. **Method.** A cross-sectional study using data from medical records of AMI patients. The spectrum of AMI includes ST-segment elevation myocardial infarction (STEMI) or non-STEMI. Data analysis was performed using SPSS 20. **Results.** A total of 136 patients were included, 72.1% male with mean age  $56.1 \pm 10.5$  years. NLR ( $p < 0.001$ ) and PLR ( $p < 0.001$ ) among GRACE risk score groups were significantly different. Mean NLR in low, intermediate, and high GRACE risk score groups were  $3.5 \pm 2.4$ ,  $6.0 \pm 4.8$ , and  $7.9 \pm 3.9$ , respectively. Mean PLR in low, intermediate, and high GRACE risk score groups were  $117.2 \pm 62.3$ ,  $183.7 \pm 95.8$ , and  $209.9 \pm 83.4$ , respectively. NLR ( $r = 0.527$ ;  $p < 0.001$ ) and also PLR ( $r = 0.496$ ;  $p < 0.001$ ) was significantly positively correlated with GRACE risk score. **Conclusion.** NLR and PLR are simple and cost-effective inflammatory markers to predict a GRACE risk score and an additional prognostic tool in AMI.

**Keywords:** AMI, GRACE risk score, NLR, PLR

### ABSTRAK

**Latar Belakang.** Rasio neutrofil-limfosit (RNL) dan rasio platelet-limfosit (RPL) merupakan petanda inflamasi penyakit kardiovaskular baru. Skor risiko *Global Registry of Acute Coronary Events* (GRACE) digunakan untuk prediksi risiko mortalitas sindrom koroner akut. **Tujuan.** Menyelidiki hubungan antara RNL dan RPL dengan skor risiko GRACE pada pasien infark miokard akut (IMA). **Metode.** Studi potong lintang atas data rekam medis pasien IMA. Spektrum IMA meliputi IMA dengan Elevasi Segmen ST (IMA-EST) dan IMA Non-Elevasi Segmen ST (IMA-NEST). Analisis data menggunakan SPSS 20. **Hasil.** Sebanyak 136 pasien diteliti, 72,1% laki-laki dengan usia rerata  $56,1 \pm 10,5$  tahun. Terdapat perbedaan signifikan nilai RNL ( $p < 0,001$ ) dan RPL ( $p < 0,001$ ) di antara kelompok skor risiko GRACE. Rerata RNL kelompok skor risiko GRACE rendah, sedang, dan tinggi adalah masing-masing  $3,5 \pm 2,4$ ;  $6,0 \pm 4,8$ ; dan  $7,9 \pm 3,9$ . Rerata RPL kelompok skor risiko GRACE rendah, sedang, dan tinggi adalah masing-masing  $117,2 \pm 62,3$ ;  $183,7 \pm 95,8$ ; dan  $209,9 \pm 83,4$ . RNL memiliki korelasi positif signifikan dengan skor risiko GRACE ( $r = 0,527$ ;  $p < 0,001$ ). RPL juga memiliki korelasi positif signifikan dengan skor risiko GRACE ( $r = 0,496$ ;  $p < 0,001$ ). **Simpulan.** RNL dan RPL merupakan petanda inflamasi sederhana dan hemat biaya yang dapat memprediksi skor risiko GRACE dan memberikan nilai prognostik tambahan pada IMA. **Herick Alvenus Willim, Simon Prayogi Pakpahan, Elnath Suprihatin, Andini Agustyana, Dwisetyo Gusti Arilaksono, Suhardi.** Hubungan Antara Rasio Neutrofil-Limfosit (RNL) dan Rasio Platelet-Limfosit (RPL) dengan *Global Registry of Acute Coronary Events Risk Score* pada Infark Miokard Akut

**Kata kunci:** IMA, RNL, RPL, skor risiko GRACE

### BACKGROUND

Cardiovascular diseases (CVD) are the leading global causes of death.<sup>1</sup> Atherosclerosis is the primary cause; rupture of vulnerable atherosclerotic plaque followed by thrombus formation leads to occlusion of the affected coronary artery causes necrosis of myocardial tissue. These events are clinically referred to as acute coronary syndrome (ACS), which

includes ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP).<sup>2</sup>

Increased local and systemic inflammation plays a significant role in the pathophysiology of ACS.<sup>3,4</sup> Neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR)

are novel inflammatory biomarkers that have been extensively investigated in recent years and are associated with adverse cardiovascular outcomes.<sup>5,6</sup> Neutrophils are recognized as crucial players in the process of athero-inflammation. Activated neutrophils are significant contributors to arterial thrombosis after plaque rupture.<sup>7</sup> In contrast, lymphocytes regulate the inflammatory response and have

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an antiatherosclerotic role.<sup>8</sup> Low lymphocyte count is a common finding during a systemic inflammatory response. It is associated with worse ACS patients.<sup>9</sup> High NLR is associated with lower ejection fraction, in-hospital complications, and higher mortality rate in acute myocardial infarction (AMI).<sup>10</sup>

Platelets have a critical role in inflammation response.<sup>11</sup> Increased platelet activation plays a significant role in the progression of atherosclerosis. Elevated platelet counts reflect increased platelet activation, which plays a significant role in megakaryocytic proliferation and produces relative thrombocytosis. High PLR was found to be a predictor of all-cause mortality and cardiovascular events.<sup>12</sup>

The Global Registry of Acute Coronary Events (GRACE) risk score is a scoring system to help clinicians determine ACS mortality risk. Patients can be categorized as low, intermediate, or high risk based on the GRACE risk score.<sup>13</sup> This study investigates the association of NLR and PLR with GRACE risk score in AMI patients.

### METHOD

A retrospective study with a cross-sectional design using medical records data from RS Pupuk Kaltim, Bontang, East Borneo, Indonesia. Patients with AMI diagnosis (including STEMI or NSTEMI) from January 2014 to December 2019 were included in this study. Exclusion criteria were clinical evidence of acute infection, current inflammatory condition of other reasons than AMI, cancer, hematological disease, autoimmune disease, chronic liver disease, and chronic kidney disease. Demographic data and variables were obtained from medical records. Variables include complete blood count (neutrophils, lymphocytes, platelets) at emergency department admission and data for GRACE risk score.

Diagnosis of AMI was based on the presence of acute myocardial injury detected by a rise and/or fall of cardiac troponin (cTn) values above the 99<sup>th</sup> percentile upper reference limit (URL) along with the presence of characteristic symptoms and/or new electrocardiographic (ECG) findings of acute myocardial ischemia (including ST-elevation, ST-depression, T-inversion, new bundle branch block).<sup>14</sup> AMI spectrum includes both STEMI and NSTEMI.<sup>14</sup>

**Table 1.** Characteristics of the study population (n=136)

Variable	Value
Age, years	56.1 ± 10.5
Gender	
Male, n (%)	98 (72.1%)
Female, n (%)	38 (27.9%)
AMI	
STEMI, n (%)	67 (49.3%)
NSTEMI, n (%)	69 (50.7%)
GRACE Risk Score	
Low Risk, n (%)	74 (54.4%)
Intermediate Risk, n (%)	37 (27.2%)
High Risk, n (%)	25 (18.4%)
NLR (mean ± SD)	4.9 ± 3.9
PLR (mean ± SD)	152.3 ± 85.7

**Table 2.** Comparison of NLR among GRACE risk score groups using one-way ANOVA test

GRACE Risk Score	n	NLR (Mean ± SD)	P Value
Low Risk	74	3.5 ± 2.4	<0.001
Intermediate Risk	37	6.0 ± 4.8	
High Risk	25	7.9 ± 3.9	

**Table 3.** Comparison of PLR among GRACE risk score groups using one-way ANOVA test

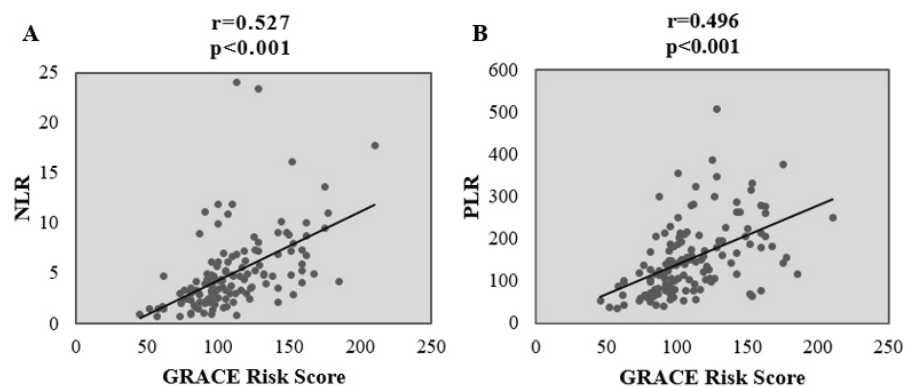
GRACE Risk Score	n	PLR (Mean ± SD)	P Value
Low Risk	74	117.2 ± 62.3	<0.001
Intermediate Risk	37	183.7 ± 95.8	
High Risk	25	209.9 ± 83.4	

**Table 4.** Post-hoc test comparison of NLR among GRACE risk score groups

	Mean Difference	95% Confidence Interval		P Value
		Minimum	Maximum	
High vs. intermediate risk	1.9	-0.8	4.7	0.230
High vs. low risk	4.4	2.3	6.5	<0.001
Intermediate vs. low risk	2.5	0.42	4.6	0.014

**Table 5.** Post-hoc test comparison of PLR among GRACE risk score groups

	Mean Difference	95% Confidence Interval		P Value
		Minimum	Maximum	
High vs. intermediate risk	26.2	-30.2	82.7	0.591
High vs. low risk	92.6	46.9	138.3	<0.001
Intermediate vs. low risk	66.4	23.6	109.2	0.001



**Figure A:** Correlation between NLR and GRACE risk score; **B:** Correlation between PLR and GRACE risk score



NLR was calculated by dividing the neutrophil count by lymphocyte count. PLR was calculated by dividing the platelet count by lymphocyte count.<sup>4</sup> GRACE risk score was calculated by using specific variables collected at admission (age, heart rate, systolic blood pressure, creatinine, presence of cardiac arrest on admission, ST-segment deviation, abnormal cardiac enzymes, and heart failure based on Killip classification), classified as low ( $\leq 108$ ), intermediate (109-140), or high risk ( $\geq 141$ ).<sup>15</sup>

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) v.20. Qualitative data were expressed as frequencies along with percentages. Quantitative data were expressed in the form of mean  $\pm$  standard deviation. The Kolmogorov-Smirnov test of normality was used to assess the normal distribution of quantitative variables. A one-way ANOVA test was used to evaluate the significant difference between NLR and PLR among GRACE risk score groups. If the results were significant, a posthoc test was used to identify where the significant differences occurred. Correlations between NLR and PLR with GRACE risk score were assessed with Pearson's correlation test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Baseline Characteristics

The study population consisted of 136 AMI patients (Table 1), 67 (49.3%) were STEMI, and 69 (50.7%) were NSTEMI. In all, 98 (72.1%) of the patients were male, with a mean age of  $56.1 \pm 10.5$  years. According to the GRACE risk score, 74 (54.4%) patients had low risk, 37 (27.2%) patients had intermediate-risk, and 25 (18.4%) patients had high risk. Mean NLR was  $4.9 \pm 3.9$ , and mean PLR was  $152.3 \pm 85.7$ .

### Comparison of NLR and PLR Among GRACE Risk Score Groups

Based on the normality test using Kolmogorov-Smirnov, all continuous variables showed normal distribution. A one-way ANOVA test was performed to compare NLR and PLR among GRACE risk score groups (low, intermediate, and high risk). The result was a significant NLR difference among GRACE risk score groups ( $p < 0.001$ ) (Table 2) and also a significant PLR difference among GRACE risk score groups ( $p < 0.001$ ) (Table 3).

Post-hoc Tamhane test found a significant difference of NLR between the high and low GRACE risk score group ( $p < 0.001$ ) and between the intermediate and low GRACE risk score group ( $p = 0.014$ ) (Table 4). There was also a significant difference in PLR between the high and low GRACE risk score group ( $p < 0.001$ ) and between the intermediate and low GRACE risk score group ( $p = 0.001$ ) (Table 5).

### Correlation Between NLR and PLR with a GRACE Risk Score

Pearson correlation test revealed a significant positive correlation between NLR and GRACE risk scores ( $r = 0.527$ ;  $p < 0.001$ ), also a significant positive correlation between PLR and GRACE risk scores ( $r = 0.496$ ;  $p < 0.001$ ) (Figure). These positive correlations mean that higher NLR and/or PLR will be followed by higher GRACE risk score.

## DISCUSSION

Inflammation plays a significant role at all stages of atherosclerosis, from the initiation, progression, and plaque destabilization. Acute inflammatory response during AMI plays a role in cardiac repair.<sup>16</sup> However, excessive inflammatory response leads to adverse left ventricular remodeling and heart failure (HF).<sup>16</sup> Besides local inflammation, profound systemic inflammation response also present in AMI.<sup>16</sup>

NLR and PLR have recently been proposed as critical inflammatory markers and potential predictors of risk and prognosis in CVDs.<sup>8,17</sup> Chen, *et al*, demonstrated that high NLR ( $> 4.0$ ) was associated with increased myocardial damage and cardiac dysfunction in ACS patients.<sup>18</sup> Zhou, *et al*, showed that high PLR ( $> 171$ ) was associated with more severe coronary artery stenosis and a higher rate of major adverse cardiac events in CAD patients.<sup>19</sup> GRACE risk score is a validated scoring system to estimate in-hospital and 6-month mortality among ACS patients.<sup>20</sup>

This study showed that both NLR and PLR were positively correlated with the GRACE risk score. This positive correlation means that higher NLR and/or PLR is correlated to the higher GRACE risk score. These results were similar to previous studies. Oncel, *et al*, demonstrated a significant positive correlation between NLR and GRACE risk score in STEMI patients ( $r = 0.803$ ,  $p < 0.001$ ).<sup>21</sup> Zhou, *et*

*al*, showed a significant positive correlation between PLR and GRACE risk score in ACS patients ( $r = 0.190$ ,  $p < 0.001$ ).<sup>22</sup>

In response to inflammation stimulus, leukocytes release many inflammatory cytokines, such as TNF- $\alpha$ , IL-6, CRP, and some proteolytic enzymes. These inflammatory cytokines have destructive effects on the myocardium.<sup>23</sup>

Neutrophils are the first leukocytes to be found in the damaged area during AMI and are seen as markers of ongoing inflammation.<sup>18</sup> Neutrophilia is associated with death and HF post-MI.<sup>24</sup> Activated neutrophils called polymorphonuclear cells (PMNs) were highly activated in coronary thrombus in patients with STEMI who were undergoing primary percutaneous coronary intervention. PMNs release neutrophil extracellular traps (NETs) at the culprit lesion, which can propagate thrombosis. NETs correlated positively with infarct size and negatively with ST-segment resolution.<sup>25</sup> Lymphocytes, especially T cells, serve to protect against adverse ventricular remodeling and improve cardiac function after AMI via inhibition of inflammation and direct protection of cardiomyocytes.<sup>26</sup> Lymphopenia is associated with a greater long-term risk of death after AMI. In the setting of AMI, lymphopenia is potentially caused by an increased level of corticosteroids and increased lymphocyte apoptosis in the context of uncontrolled immune system activation.<sup>27</sup> Platelets play a role in developing atherosclerosis by producing inflammatory mediators and directing leukocyte aggregation into plaques through platelet-mediated leukocyte adhesion.<sup>28</sup> High platelet count was associated with a higher rate of adverse clinical outcomes in AMI, like HF, arrhythmia, re-infarction, and death.<sup>29</sup>

Our study has demonstrated that NLR and PLR were significantly and positively correlated with the GRACE risk score. It can be used as additional predictors of AMI outcome besides GRACE risk score. Elevated NLR signifies the risk of increased neutrophil and decreased lymphocyte, whereas high PLR integrated the risk of increased platelet and decreased lymphocyte. Hence, it should be more predictive than neutrophil, platelet, and lymphocyte count alone.



### CONCLUSION

NLR and PLR are simple, cost-effective, and useful inflammatory markers that can predict the increase of GRACE risk score and provide additional prognostic value in AMI patients.

### Study Limitation

This study was a retrospective and single-center study. Further studies with larger samples and multicenter are required to substantiate the present findings.

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