

Turnitin-Correlation between platelet lymphocyte ratio

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Correlation between Platelet to Lymphocyte Ratio with C-Reactive Protein in COVID-19 Patients

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ABSTRACT

Coronavirus 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Inflammation occurs when the body is infected with the virus. Platelets play a role in immune response and immunomodulation by activating P-Selectin Glycoprotein (PSGL) to the site of inflammation. Lymphocytes play a role through CD4 T-cells, B-cells producing specific viral antibodies, and CD8 cytotoxic T-cells by directly killing the virus in infected cells. This study aimed to prove the correlation between PLR and CRP as inflammation markers in COVID-19 patients. This study was a retrospective observational study with the cross-sectional approach at Dr. Kariadi Hospital, Semarang, for the period March-August 2020. Spearman test performed for analyzing data with $p < 0.05$ was significant. Thirty-three confirmed COVID-19 patients with median value of PLR 218 (103-1609) and CRP 15.94 (1.24-200) mg/L were tested for correlation with a value of $p = 0.013$ dan $r = 0.427$. The increase of PLR and CRP in COVID-19 patients was caused by an inflammatory process mediated by the immune response. High values in the blood were associated with disease severity and poor prognosis. There was a statistically significant moderate positive correlation between PLR and CRP in COVID-19 patients.

Keywords: Platelet to lymphocyte ratio, C-reactive protein, COVID-19

INTRODUCTION

Coronavirus 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The nature of this virus is zoonotic (transmitted between animals and humans). However, the particular animal as the source of transmission of COVID-19 is still unknown.¹

The first COVID-19 case was found in Wuhan City, Hubei Province, China, marked by the first reported case of mysterious pneumonia on December 31, 2019. On January 30, 2020, the World Health Organization (WHO) declared the incident as a Public Health Emergency of International Concern (PHEIC), and on March 11, 2020, WHO declared COVID-19 as a pandemic. This virus spread rapidly and to various countries in a short time. As of July 9, 2020, WHO reported 11,840,226 confirmed cases with 545,481 deaths worldwide (Case Fatality Rate/CFR 4.6%). In Indonesia, the existence of the virus was first known in March, and according to data from the Ministry of Health, has exceeded 70,736 cases with 3,417 deaths (CFR 4.8%) as of July 9, 2020.^{1,2}

Common symptoms of COVID-19 infection include acute respiratory problems such as fever, cough, and shortness of breath. Severe cases of COVID-19 can cause pneumonia, acute respiratory syndrome, kidney failure, and even death. The average incubation period is 5-6 days, with the most extended incubation period of 14 days. Inflammation caused by the transmission of infectious diseases plays a vital role in the development of the virus. A severe inflammatory response contributes to a weak adaptive immune response resulting in an immune response imbalance that inhibits interactions with blood cells. Interaction with blood cells is critical in inflammation, immune response, hemostasis, and oncogenesis.¹ Platelet to lymphocyte ratio (PLR) can describe systemic inflammation used in various diseases such as acute coronary syndrome, cancer, chronic kidney disease, and others.³⁻⁵ Another laboratory marker of inflammatory response is C-Reactive Protein (CRP), which has been identified as a predictor of clinical complications and severity. C-reactive protein is a marker of acute phase

inflammation that has been found to be associated with disease severity and response therapy. Several studies stated that PLR and CRP are markers of acute inflammation, used in various diseases to see prognosis and mortality.⁶⁻⁸ A study by Kartal *et al.* stated that 114 patients with pneumonia showed an increase in PLR and CRP levels compared to the control group. This is related to the occurrence of inflammation and can be used as a diagnostic marker of pneumonia.⁶

This study intended to find out the correlation between these two parameters (PLR and CRP) in positive patients for COVID-19. The result of this study was expected to be used as a sign of acute inflammation and a predictor of patients experiencing clinical deterioration to minimize the risk of patients. This study aimed to prove a relationship between PLR and CRP in COVID-19 patients.

METHODS

This retrospective observational study was conducted with a cross-sectional approach in March-August 2020 at Dr. Kariadi Hospital, Semarang. The research data were taken from the medical records of patients who were admitted and confirmed positive for COVID-19 by consecutive sampling on one occasion. The types of data used were secondary data such as patients' characteristics. The calculation of the PLR value was obtained by calculating the number of platelets divided by absolute lymphocytes, and CRP levels were measured with the immunoturbidimetry method. The inclusion criteria included male and female patients aged 30-65 years with positive swab/PCR results. The exclusion criteria were children and pregnant females, history of

malignancy, radiation therapy/chemotherapy, autoimmunity, and HIV.

The sample size was 33 people according to a predetermined formula. The data was processed using the IBM SPSS statistics. The data normality test was analyzed using the Shapiro-Wilk test and then analyzed using Spearman's correlation test. Data were presented as mean and standard deviation. The p-value of < 0.05 is considered statistically significant.

The Medical and Health Research Ethics Committee, Dr. Kariadi Hospital, Semarang, approved this research with Number 604/EC/KEPK-RSDK/2020.

RESULTS AND DISCUSSIONS

The study was conducted on 33 patients with a final diagnosis of COVID-19 who had tested positive for swab/PCR at Dr. Kariadi Hospital and fulfilled both inclusion and exclusion criteria. The proportion of the research subjects were dominated by female, consisting of 11 male (33.3%) and 22 female (66.7%). The respondent's median, minimum and maximum age was 53 (35-76) years old (Table 1).

After being analyzed using the Shapiro-Wilk test, p < 0.001 was obtained, suggesting abnormal distribution for platelet and PLR. The results of Spearman's analysis, p=0.013 and r=0.427, showed a moderate positive correlation between PLR and CRP with the p-value of < 0.05, which illustrates that there was a significant correlation (Table 2 and Figure 1).

Table 2. The results of Spearman's PLR correlation test to CRP

Variable	CRP	
	r	p
PLR	0.427	0.013*

Spearman's test, *p < 0.05

Table 1. General characteristics of research subjects

Variable (n=33)	F	%	Mean±SD	Median (min-max)
Gender				
Male	11	33.3		
Female	22	66.7		
Age (year)			53.27±9.85	53 (35-76)
Hemoglobin (g/dL)			11.95±2.69	12.1 (6.2-17.2)
Hematocrit (%)			35.90±7.90	36.2 (19.2-52.6)
Leukocyte (x10 ³ /μL)			12.97±6.34	11.8 (2.9-26.1)
Platelet (x10 ³ /μL)*			278,73±146,99	234 (34-738)
PLR*			323,65±294,08	218 (103-1609)
CRP (mg/L)			33.75±42.48	15.94 (1.24-200)

Note: SD (Standard Deviation); min (minimal); max (maximum), *Data distribution is not normal; PLR (Platelet to Lymphocyte Ratio); CRP (C-Reactive Protein)

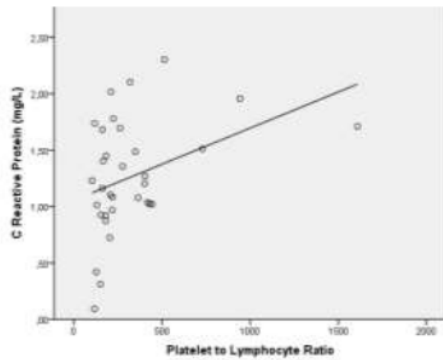


Figure 1. Scatter plot between PLR and CRP levels

The transmission of SARS-CoV2 is by droplets through coughing, sneezing, or direct contact with infected individuals. The virus enters the mucous membrane of the host's cell, travels into the respiratory tract to the alveoli in the lungs. In the alveoli, the virus binds specifically to the Angiotensin-Converting Enzyme 2 (ACE2) receptor in type II pneumocytes and replicates. Common symptoms of COVID-19 include acute respiratory distress symptoms such as fever, cough, and shortness of breath. In cases of COVID-19, it can develop into pneumonia, acute respiratory syndrome, kidney failure, coagulation disorder, and in some patients with severe inflammation or cytokine storms, it can cause death. Several studies regarding COVID-19 have similarities in symptoms and laboratory findings with SARS-Cov, where patients showed lymphopenia, thrombocytopenia, and leucopenia.⁹ Therefore, it is crucial to see the progress of biological markers to predict clinical deterioration and minimize the risk of death for COVID-19 patients so that optimal management can be given.

Platelets are cells in the human body that play an important role in hemostasis, coagulation, angiogenesis, immune response, inflammatory response, and biologic tumor. Changes in platelet count and activity are associated with various diseases.^{8,10,11} Platelets are produced by megakaryocytes in the bone marrow, and various cytokines such as TPO, IL-3, IL-6, IL-9, IL-11, and Stem Cell Factor (SCF) can increase the production of megakaryocytes. For example, in an inflammatory state, IL-6 can promote the generation of megakaryocytes by stimulating an increase of the TPO level.¹²

Platelets also contain three secretory granules. There are α -granules, dense granules, and lysosomal granules, one of which plays a role in the pathogen clearance mechanism by regulating the activity of immune cells such as neutrophils and activation of P-Selectin Glycoprotein Ligand-1 (PSGL-1, CD162) to the site of inflammation.¹³ P-selectin is synthesized by megakaryocytes and endothelial cells residing in α -granules and Weibel-Palade bodies. When stimulated, selectin will move to the plasma membrane to mediate leukocyte rolling, the initial process of the leukocyte adhesion cascade. It was stated in several previous studies that platelets can affect various cells such as neutrophils, T-lymphocytes, phagocytes, endothelial cells, and dendritic cells.¹⁴ Study by Koupenova *et al.* TLR7 on platelets is the Pattern Recognition Receptor (PRR) is involved in the initial recognition of influenza virus ssRNA. In addition, platelets also contain C3 in their granules. There was an increased level of C3 during infection in-vivo and also in circulation compared to controls.¹⁰ This shows that platelets will not only migrate to the site of inflammation but also actively move to the damaged tissue; platelets will form extracellular traps, phagocytosis, and clearance of pathogens such as neutrophils in the recognition mechanism TLR.

T-cells, namely CD4⁺ and CD8⁺ cells, play an essential role in balancing antivirus to fight pathogens and reduce the risk of autoimmunity or excessive inflammation. CD4⁺ T-cells will produce specific viral antibodies by activating B-cells. CD8⁺ T-cells are cytotoxic T-cells that will kill the virus directly in infected cells. Approximately 80% of CD8⁺ T-cells are present in the lungs infected with SARS-Cov2 and play a role in the mechanism of cleaning cells infected with the virus and suppressing immune reactions. The decrease in the number of CD4⁺ T-cells is closely related to the mobilization of lymphocytes in the lungs due to neutralizing antibodies and the production of cytokines. Helper T-cells also produce pro-inflammatory cytokines through the NF- κ B pathway.^{15,16} This triggers a cytokine storm in the body and delivers a variety of immune responses that cause changes in peripheral leukocyte count.¹⁷ Persistent lymphopenia can increase viral replication, partly explaining the relationship

between low lymphocyte count and poor outcome.

Platelet to lymphocyte ratio is a comparison between platelet and absolute lymphocyte count. It is important to understand the role of platelets and lymphocytes in COVID-19. The results showed that PLR was associated with CRP ($r=0.427$; $p=0.013$) in COVID-19 patients. This can be interpreted that PLR and CRP play a role in the inflammatory and immunomodulatory processes. In line with Abigail and Daniel *et al.*, high PLR values were more common in COVID-19 patients with severe symptoms than patients without them. The possibility of a high PLR value in severe symptomatic cases is due to a low absolute lymphocyte value compared to a decrease in platelet count.^{18,19} This study did not differentiate between COVID-19 patients with severe symptoms, but there was an increase in PLR 218 (103-1609), which might show severe symptoms in some patients. A decrease in platelet count in COVID-19 patients can be caused by: Suppression of bone marrow hematopoietic cell or bone marrow stromal cell; Increased autoantibodies and immune complex resulting in increased platelet destruction; Lung damage leading to activation, aggregation, and formation of microthrombus resulting in thrombocytopenia; Pulmonary capillary damage leads to rupture of megakaryocyte and inhibits platelet release.²⁰ Decrease in absolute lymphocyte count may occur due to the role of pro-inflammatory cytokine as previously described, and decreased lymphocyte is associated with poor prognosis in COVID-19 patients. A study by Que *et al.* found that PLR can determine disease progression in COVID-19 patients to prevent severe complications such as cytokine storms. PLR can also predict the prognosis and mortality of patients during treatment.⁸

The production of pro-inflammatory cytokines such as IL-6 and other cytokines can promote the synthesis of an inflammatory protein in the liver, such as CRP. Hence the CRP levels in the blood of COVID-19 patients will increase as an acute-phase inflammatory response in the pathogen clearance mechanism through the activation of the classical complement pathway and phagocytosis and the opsonization of dead cell or tissue.²¹ In this study, there was an increase in CRP level with a median

of 15.94 (1.24–200), normally found at a concentration of less than 10 mg/L in the blood. This is consistent with the pathogenesis of COVID-19, where there is damage to various organs due to excessive inflammatory response.^{22,23} In line with this study, there was an increase in PLR and CRP as predictor severity of symptoms. Research done by Liu *et al.* who stated that the relationship between serum IL-6 levels and CRP could effectively predict disease severity and outcome in COVID-19 patients, CRP value is higher in cases of patients with severe clinical symptoms compared to those with mild to moderate clinical symptoms by showing high diagnostic value for clinical severity.²⁴

CONCLUSIONS AND SUGGESTIONS

Based on the study results, there was a moderate positive correlation between PLR and CRP in COVID-19 patients. Platelet to lymphocyte ratio can be used as a marker of inflammation in addition to CRP in COVID-19 patients. However, this study did not differentiate between COVID-19 patients with severe symptoms and those without severe symptoms. It is necessary to do further research involving a more significant number of samples.

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