

Analysis of Platelet Lymphocyte Ratio on Severe COVID-19 and Dengue Hemorrhagic Fever Grade IV

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ABSTRACT

Thrombocytopenia can occur in severe COVID-19 or grade IV DHF. In COVID-19, lymphopenia occurs gradually due to Angiotensin Converting Enzyme-2 as the primary response to SARS-CoV-2 and cytokines that can damage lymphocytes. Contrastingly, lymphocytosis occurs in dengue virus infection. Platelet-to-lymphocyte ratio (PLR) is a new inflammatory marker in predicting the severity of both diseases. This study aimed to analyze differences in PLR values in severe COVID-19 and grade IV DHF and determine the cut-off values. Retrospective research of a cross-sectional approach was carried out using medical record data of patients diagnosed with severe COVID-19 and grade IV DHF by clinicians at Dr. Wahidin Sudirohusodo Hospital from January 2017 to October 2021. The platelet-to-lymphocyte ratio was obtained from the complete blood count results using the flow cytometry method at the beginning of hospitalization. Mann-Whitney test and Receiver Operating Characteristics (ROC) curve were used for statistical analysis. A total of 74 severe COVID-19 and 33 grade IV DHF patients were involved; PRL of severe COVID-19 in dead patients was higher than in recovered patients ($p > 0.05$). The platelet-to-lymphocyte ratio value was significantly lower in grade IV DHF patients who died compared to those who recovered ($p < 0.001$). Platelet to-lymphocyte ratio cut-off of 48.30 for severe COVID-19 was obtained with an Area Under the Curve (AUC) of 0.631 (sensitivity of 54.3%, specificity of 78.6%) and cut-off of 0.715 for grade IV DHF with an AUC of 0.989 (sensitivity of 94.4%, specificity of 100%). There was a difference in PLR value in severe COVID-19 and grade IV DHF. Severe COVID-19 patients who died had higher PLR, whereas grade IV DHF patients had lower PLR values. Values above the PLR cut-off of 48.30 for severe COVID-19 and 0.715 for grade IV DHF can predict the severity of the disease.

Keywords: Platelet to-lymphocyte ratio, severe COVID-19, DHF grade IV

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), transmitted by droplet and direct contact. This virus targets the respiratory system and leads to acute pneumonia. Patients with severe degrees of COVID-19 can experience worsening disease, sepsis, multi-organ failure, and death. Based on data from the World Health Organization and PUSDATIN Ministry of Health, the number of confirmed cases of COVID-19 as of July 2022 has reached 555,446,890 people worldwide and has resulted in the death of more than 6,353,692 people. Confirmed cases of COVID-19 in Indonesia have reached 6,120,169 people with 156,818 deaths, whereas its cases in South Sulawesi have reached 143,680 people with 2,477 deaths.¹⁻³

Indonesia is one of the tropical countries where dengue is endemic. Dengue Hemorrhagic Fever (DHF) is a viral infection that is transmitted through the bite of female *Aedes aegypti* and *Aedes*

albopictus mosquitoes. In the era of the COVID-19 pandemic, COVID-19 and DHF dengue cases were increasing, and the co-infection of COVID-19 and DHF was common, indicating a need to avoid any error in diagnosis. This fact remains challenging for clinicians because these two diseases show similar viral antigen structures, clinical symptoms, and laboratory findings, making it difficult to distinguish both diseases. Thrombocytopenia due to endothelial dysfunction is similar to laboratory results of severe COVID-19 and grade IV DHF.^{4,5}

Platelet Lymphocyte Ratio (PLR) is a new inflammatory marker that can be used to predict disease severity. Platelet lymphocyte ratio is calculated from the Complete Blood Count (CBC), which is a simple, rapid, inexpensive, and more clinically meaningful test compared to platelet or lymphocyte count as the only parameter used. Complete blood count results of DHF are characterized by a progressive increase in leukocytes followed by thrombocytopenia and an increase in hematocrit. Nisa found that 97.2% of DHF patients experienced thrombocytopenia and experienced

significant plasma leakage accompanied by increased permeability. A lower platelet count in DHF leads to a higher mortality rate. Sendy *et al.* found a significant relationship between lymphocyte count and the severity of DHF patients, indicating that a higher lymphocyte count would lead to a more severe disease. Contrastingly, leukopenia/leukocytosis, eosinopenia, and lymphopenia in COVID-19 may occur as the most common abnormalities, which can be used as valuable indicators to determine the clinical severity of the disease.^{6,7}

Inflammation plays a significant role in the pathophysiology of cytokine storms as a characteristic of severe COVID-19 disease. The study by Shen *et al.* showed that PLR plays an essential role in the inflammatory process and as a predictor of mortality. A study by Martin *et al.* found that six studies in China showed the same results; an increase in the PLR value at admission was found in cases of severe COVID-19. In addition, significantly decreasing PLR was found in a critical phase of DHF.⁸⁻¹¹

Based on the description above, it is presumed that there are differences in PLR values in patients with COVID-19 and DHF, although viruses cause both diseases. Therefore, the authors are interested in analyzing the differences in PLR values and determining the cut-off values in patients with severe COVID-19 and grade IV DHF against disease severity. In addition, the parameter that has been frequently studied recently is the neutrophil-lymphocyte ratio in patients with COVID-19 and DHF, whereas research on PLR in severe COVID-19 associated with grade IV DHF in Indonesia is still rare, especially in Makassar. No studies have been published regarding PLR values in severe COVID-19 and grade IV DHF.

METHODS

This research was a retrospective study with a cross-sectional method using secondary data from

patient medical records from January 2017 to October 2021. The research was conducted at the Medical Records Installation of Dr. Wahidin Sudirohusodo Hospital, Makassar, from September to October 2021. The population in the study were all patients confirmed to have severe COVID-19 based on the Polymerase Chain Reaction (PCR) test and grade IV DHF based on IgG and IgM tests. The sample of this study was an accessible population that met the inclusion criteria: age > 18 years, a patient diagnosed by clinicians with severe COVID-19 and grade IV DHF, and had data on CBC results. Patients with bacterial infection, malignancy, and coronary heart disease were excluded.

The samples were then statistically analyzed using SPSS version 25. The variables for gender, age, outcome, and PLR values in the COVID-19 and degree IV DHF were analyzed using the Mann-Whitney test. The age variable was analyzed using the independent T-test test, and the cut-off value of PLR was determined based on the Receiver Operating Characteristics (ROC) curve. The statistical test results were reported to be significant if the p-value < 0.05. Research permission was obtained from the Health Research Ethics Commission, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital with number 662/UN4.6.4.5.31/PP36/2021.

RESULTS AND DISCUSSIONS

The total sample for this study was 107 patients, who were divided into 74 patients with severe COVID-19 and 33 patients with grade IV DHF.

Table 1 shows the differences in the characteristics of the research subjects between groups of severe COVID-19 and grade IV DHF. Both groups had the same proportion, indicating more males than females. There was a higher number of males (59.5%) compared to females (40.5%) in the severe COVID-19 group, and there was a higher

Table 1. Characteristics of research subjects

Criteria	Severe COVID-19	Grade IV DHF
Gender (n %)		
Male	44 (59.5)	19 (57.6)
Female	30 (40.5)	14 (42.4)
Age (Mean±SD)	50 ± 19.07	36 ± 13.12
Platelet 10 ³ /μL (median (min-max))	256 (10–872)	20 (1–67)
Lymphocyte 10 ³ /μL (median (min-max))	7.4 (2.0–50.4)	30 (8.9–76.7)
PLR (median (min-max))	31.21 (0.33– 290.66)	0.83 (0.03–2.69)
Outcome (n %)		
Recovery	28 (37.8 %)	18 (54.5 %)
Death	46 (62.2 %)	15 (45.5 %)

number of males (57.6%) compared to females (42.4%) in the grade IV DHF group. The average age of the patients suffering from severe COVID-19 and grade IV DHF was 50 and 36, respectively.

The average platelet count in grade IV DHF was lower than in the severe COVID-19 group. Platelet count in severe COVID-19 ranged from 10 to 872 $10^3/\mu\text{L}$ with an average of 256 $10^3/\mu\text{L}$, whereas platelet count in degree IV DHF ranged from 1 to 67 $10^3/\mu\text{L}$ with an average of 20 $10^3/\mu\text{L}$. The average lymphocyte count was lower in severe COVID-19 than in grade IV DHF. It was found that lymphocyte count in severe COVID-19 ranged from 2.0 to 50.4 $10^3/\mu\text{L}$ with an average of 7.4 $10^3/\mu\text{L}$, whereas lymphocyte count in grade IV DHF ranged from 8.9 to 76.7 $10^3/\mu\text{L}$ with an average of 30 $10^3/\mu\text{L}$. The average PLR value in severe COVID-19 was higher than in low-grade DHF. It was found that the PLR in severe COVID-19 ranged from 0.33 to 290.66 with an average of 31.21, whereas PLR in grade IV DHF ranged from 0.03 to 2.69 with an average of 0.83.

According to the outcome, it was found that death was more common (62.2%) compared to recovery (37.8%) in severe COVID-19 group, whereas recovery was more common (54.5%) compared to death (45.5%) in grade IV DHF group in this study.

Table 2 shows the difference in PLR values based on the patient outcomes (recovery and death). The mean PLR value of recovered and dead patients with severe COVID-19 was 19.56 and 55.31, respectively. The Mann-Whitney test results showed no

significant difference in the PLR value between recovered and deceased patients in the severe COVID-19 group, with a p-value of 0.060 ($p > 0.05$).

Based on the patient outcomes, The mean PLR value of recovered and dead patients in grade IV DHF was 1.2 and 0.19, respectively. The Mann-Whitney test results showed a significant difference in the PLR value between recovered and died patients in grade IV DHF with a p-value < 0.001 (< 0.05).

Figure 1 shows the cut-off value of PLR for the patient outcome in the severe COVID-19 group. Based on the coordinates of the ROC curve, the AUC of 0.631 and a cut-off value of 48.30 (54.3% sensitivity and 78.6% specificity) were obtained. In addition, based on the coordinates of the ROC curve, the AUC of PLR value for the patient outcome in grade IV DHF was 0.989 with a cut-off value of 0.715 (94.4% sensitivity and 100% specificity).

This study found that there were more males than females in severe COVID-19 and grade IV DHF groups. This result was in line with a meta-analysis by Zhu *et al.* on 3062 COVID-19 cases at Chinese Hospitals, which found a higher number of males (56.9%) than females (43.1%). A study by Teodora on patients treated in Wuhan City showed that a higher number of males were infected with COVID-19, and the Chinese Center for Disease states that males infected with COVID-19 have a two-fold higher risk of death than females because they may be influenced by the smoking habits, which causes the lungs more susceptible to SARS-COV2 infection

Table 2. Difference in PLR value according to disease outcome

Criteria	Recovery	Death	p
	PLR (Median (Min-Max))	PLR (Median (Min-Max))	
Severe COVID-19	19.56 (2.39 – 75.56)	55.31 (0.33 – 290.66)	0.060
Grade IV DBD	1.2 (0.70 – 2.69)	0.19 (0.03 – 0.83)	< 0.001

*Mann-Whitney test

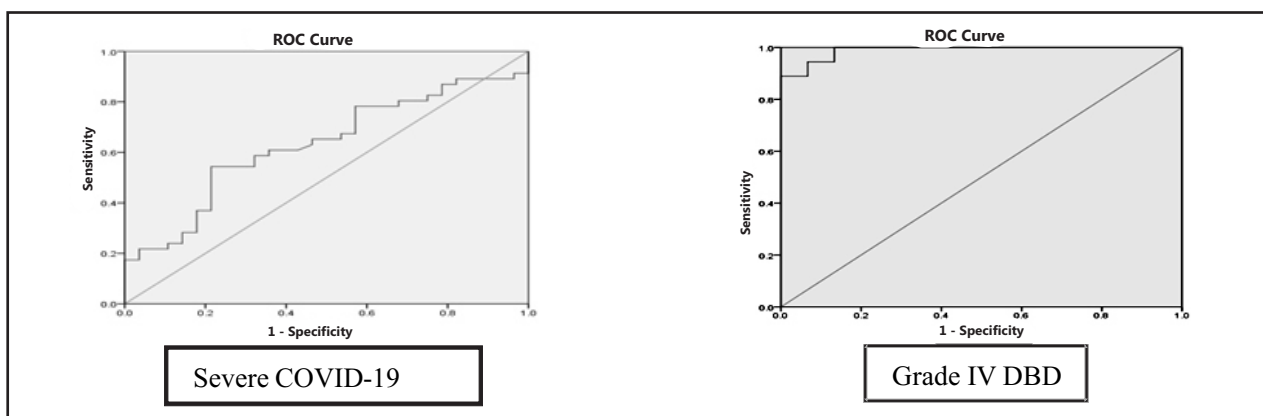


Figure 1. ROC curve of PLR value to patient outcomes in severe COVID-19 and grade IV DHF

through an increase in the Angiotensin Converting Enzyme 2 (ACE2) receptor. In addition, Hermawan states that more males are affected by DHF with dengue shock syndrome due to more efficiency of genetic and humoral antibody production in females compared to males.^{5,9}

Patients with severe COVID-19 and grade IV DHF were found to be most infected at the age of 50 and 36 years, respectively. This fact shows that patients with severe degrees of COVID-19 are infected more frequently at an older age, whereas patients with grade IV DHF are more frequently infected at a young age. A study by Liu *et al.* at the Central Hospital of Wuhan suggested that more progressive and stable cases were found at age 66 and younger, respectively. Fernando *et al.* stated that COVID-19 targets older adults, and more cases are found at age > 60 years. The older age would lead to a higher case fatality rate. Wang *et al.*, in severe cases of COVID-19, found that old age has a higher death rate after infection due to low lymphocytes count, especially CD8⁺ T-cells, which is crucial for immunity. Based on data from the Ministry of Health, DHF cases most commonly occur in the age range of 18-44 years (33.97%) because this age factor has high activity outside, increasing the risk of *Aedes aegypti* exposure and high-stress levels, thereby weakening the immune system.⁹

Platelets in grade IV DHF were lower than those in COVID-19. Thrombocytopenia is common in critically ill patients, generally with organic dysfunction/physiological compensation. In response to the dengue virus, antigen-antibody complexes cause platelet aggregation and activate the coagulation system through damage to blood vessel endothelial cells and activating the complement system. Platelet aggregation results in the release of adenosine diphosphate (ADP). It leads to platelet destruction by the reticuloendothelial system (RES), which results in thrombocytopenia. Thrombocytopenia in COVID-19 can occur in a clinical course with other severity symptoms through the cytokine storm mechanism. SARS-CoV-2 infects epithelial/immune cells, causing tissue damage and releasing inflammatory cytokines (IL-1, IL-6, IL-12, and TNF α), which then recruit innate immune cells (monocytes, macrophages, neutrophils, dendritic cells, and NK cells) and activate adaptive immune cells (CD4⁺ T-cells and CD8⁺ T-cells) to induce myelopoiesis and granulopoiesis and excessive cytokine production can exacerbate epithelial damage. Increased systemic cytokines trigger macrophage activation, causing a massive proinflammatory response that results in impaired

vascular hemostasis, leading to capillary leak syndrome, thrombosis, DIC, multi-organ failure, and death.⁷⁻⁹

Lymphocytes were found to be lower in severe COVID-19 than in grade IV DHF. This study was in line with a study by Ding *et al.* and Li *et al.*, which reported that lymphocyte count decreases gradually in COVID-19 patients because ACE2 (considered as the primary response for SARS-CoV-2) is expressed in lymphocytes and cause direct infection to cells and eventually lead to lymphopenia. The role of IL-10, IL-6, and TNF cytokines can also damage lymphocytes, causing lymphopenia. Contrastingly, increased lymphocyte count occurs in DHF. Jameel *et al.* stated that relative lymphocytosis in the presence of atypical lymphocytosis is a consistent finding in DHF cases. The mechanism of immunopathogenesis of dengue infection involves antibody production as a humoral response, which plays a role in the process of virus neutralization, complement-mediated cytolysis, antibody-mediated cytotoxicity, and involves T lymphocytes (T-helper/CD4, T-cytotoxic/CD8, monocytes, macrophages, cytokines, and complement activation).^{8,9}

This study found an average difference in PLR values of 31.21 for severe COVID-19, higher than for grade IV DHF of 0.83. The result was in line with a meta-analysis by Daniel *et al.* covering 998 COVID-19 patients, which showed that high PLR values were associated with severe COVID-19. Six of the seven included studies showed similar results, such as increased PLR upon admission found in severe COVID-19 cases. Contrastingly, a significant decrease in PLR was found in patients suffering from the critical phase of DHF.^{6,10,11}

Patients with severe COVID-19 who died had higher PLR values than those who recovered. In contrast, patients with grade IV DHF who died had significantly lower PLR values than those who recovered. Based on the ROC and AUC curve analysis, the cut-off value of PLR for the outcome (died and improved) of severe COVID-19 patients was 48.30, p-value 0.060 ($p > 0.05$), with a sensitivity of 54.3% and a specificity of 78.6%. The cut-off value of PLR for grade IV DHF patients was 0.715, $p < 0.001$ (< 0.05), with a sensitivity of 94.4% and a specificity of 100%. The results of this study indicated that the PLR value can estimate the severity of the disease. This study was in line with a study by Qu *et al.*, which reported no significant decrease in PLR value at admission under severe conditions compared to non-severe COVID-19, although PLR value at admission increased in severe COVID-19.¹⁰

This study was conducted using a retrospective

approach based on patient medical record data; therefore, the authors could not interact with patients, directly observe patient conditions, and monitor the course of the disease.

CONCLUSIONS AND SUGGESTIONS

There was a difference in PLR values between severe COVID-19 and grade IV DHF. The PLR value for severe COVID-19 was higher in patients who died than patients who recovered. Platelet lymphocyte ratio value in grade IV DHF was lower in patients who died than in patients who recovered. Platelet lymphocyte ratio values above the cut-off of 48.30 in severe COVID-19 and the cut-off of 0.715 in grade IV DHF can predict the severity of the disease.

It was suggested that PLR value can be determined regularly as a follow-up to the patient's clinical condition. It was expected that further studies would be carried out in a larger number of patients and on an equal basis to obtain better research results.

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