

Analysis of platelet indice on the severity of COVID-19 patients

Sofwan Halimi^{1*}, Endah Setyaningrum¹, Sutyarso¹, Nuning Nurcahyani¹, Hidayat²

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung
Jl. Prof. Dr. Soemantri Brodjonegoro No.1 Bandar Lampung, Lampung, Indonesia. 35145

²Installation of Clinical Pathology Laboratory, RSUD Dr. H. Abdul Moeloek Lampung
Jl. Dr. Rivai No. 6 Penengahan, Bandar Lampung, Lampung, Indonesia. 35121

*Email: sofwanhalimi@gmail.com

ABSTRACT. The cause of Covid-19 infection is the SARS-CoV-2 virus which can be detected using Real Time Reverse-transcriptase Polymerase Chain Reaction (rRT-PCR). Platelets play a role in inflammation and the pathogenesis of various inflammatory clinical conditions which are also affected by COVID-19 infection, which can change according to the severity of the infection. The aim of this study was to analyze the differences in the mean platelet volume (MPV) and platelet distribution width (PDW) indice in the severity of COVID-19 sufferers. This type of research is an analytical observational study with a cross sectional research design. Data on platelet indice values and degrees of severity were obtained from medical records during the period June to August 2021 from 209 COVID-19 sufferers treated at RSUD Dr. H. Abdul Moeloek Lampung Province. Statistical tests were carried out using the Anova Kruskal Wallis test, followed by the Mann Whitney test. The research results showed that there was a significant difference in the PDW indice ($p = 0.004$) in the severity of COVID-19, while there was an insignificant difference in the MPV indice in the degree of severity of COVID-19. The pathogenesis of SARS-CoV-2 infection is very complex, excessive production of cytokines and acute phase reactants due to the immune response and inflammation due to SARS-CoV-2 infection which affects the development of megakaryocytes results in changes in the platelet indice. The findings of this study show that the PDW platelet indice can be a predictor of the severity of COVID-19 sufferers.

Keywords: COVID-19; platelets; platelet indice; SARS-CoV-2 infection; severity of COVID-19

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INTRODUCTION

The world health organization (WHO) has designated COVID -19 as a pandemic. COVID -19 is an infectious disease caused by a virus and can be transmitted between humans due to the presence of the SARS-CoV-2 virus, as happened in China at the end of 2019 and spread to various countries. Based on searches on the official WHO website, 628,035,553 COVID-19 cases have been released confirmed and 6,572,800 deaths globally (<https://covid19.who.int/cases> accessed on 02 November 2022). Meanwhile, the Indonesian Ministry of Health reported that there were 6,507,610 confirmed cases in Indonesia with a death rate of 158,737 people (KEMENKES, 2022).

The city of Wuhan, China, where the new corona virus was discovered, was named *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2) by the International Committee for Virus Taxonomy (Patrucco *et al.*, 2020). SARS-CoV-2 is transmitted from human to other human within the incubation period of the virus after entering the body of around 3-7 days, sometimes up to 14 days. In this time period, transmission can occur through respiratory droplets containing the virus (Zhu *et al.*, 2020). In addition, the sequencing results show that 98% of the coronaviruses that infect bats and pangolins are very similar to the coronaviruses that infect humans. Therefore, this virus is better known as SARS-CoV-2 which can replicate in the lower respiratory tract and cause pneumonia which can end fatally with acute respiratory distress syndrome (ARDS) (Tay *et al.*, 2020).

Laboratory examinations play an important role in handling Covid-19, starting from screening, diagnosis, monitoring therapy, determining prognosis, to surveillance. Platelets originate from the fragmentation of the cytoplasm of megakaryocytes, large young cells in the bone marrow. Mature megakaryocytes are characterized by the endomyotic nuclear replication process and the increasing volume of plasma, so that in the end the cytoplasm becomes granular and platelets are released, each

megakaryocyte is capable of producing 3000-4000 platelets, the time from stem cell differentiation to platelet production takes around 10 days, the lifespan of platelets in peripheral blood is 7 – 10 days (Kiswari, 2014). Platelets are not only involved in hemostasis and coagulation but play an important role in innate immunity and inflammatory responses (Ozder *et al.*, 2020). The involvement of blood platelets in the inflammatory response is associated with the release of cytokines and chemokines that attract leukocytes and facilitate adhesion to the endothelium at the site of damage. During the inflammatory process, blood platelets can interact with leukocytes to form platelet-leukocyte aggregates. This binding is possible through adhesion proteins expressed on the cell surface during activation. In addition, platelets support leukocytes to fight bacterial infections through direct contact, encapsulation of bacteria, and release of reactive oxygen species and PMP (*Platelet Microbicidal Protein*) (Korniluk *et al.*, 2020).

Platelets have several indices that can be determined using a hematology analyzer consisting of platelet distribution width (PDW), mean platelet volume (MPV), Platelet – Large Cell Ratio (P-LCR) and Plateletcrit (PCT), which are indicators of platelet activation (Astuti, 2020). MPV is the average number of platelets circulating in peripheral blood, the normal MPV value is 8.4-12 fL. PDW is a measure of the diameter of platelets in peripheral blood, the normal PDW value is 10-18 fl (Prameswari, 2018). In a healthy state, the PDW indice and MPV indice are in inverse relationship with platelet count. To detect the proportion of the whole blood volume occupied by platelets is the Plateletcrit (PCT) parameter (Prameswari, 2018). Normal conditions, the PCT value is 0.22-0.24% (Astuti, 2020). P-LCR is the proportion of normal platelets measuring more than 12 fL, the normal PLCR value is 10-30% (Prameswari, 2018). P– LCR value is inversely proportional to platelet count and directly related to MPV and PDW (Babu and Basu, 2004).

New information is still needed regarding COVID-19 markers that have the potential to affect the severity of the disease, for this reason, clinicians need supporting examination parameters, especially laboratory examinations in the management of COVID-19. So it is necessary to know the relationship between the platelet indice and the severity of the disease in COVID-19 infection. So it is hoped that this will provide information and knowledge for clinicians regarding platelet index markers for the degree of disease in COVID-19 sufferers and as an illustration of the prognosis of COVID-19 sufferers so that appropriate treatment can be given.

MATERIALS AND METHODS

Study area. The research was carried out at the Regional General Hospital dr. H. Abdul Moeloek Lampung Province. The tool used is a Mindray BC6200 Hematology Analyzer. The ingredient used is Whole Blood. The reagent used to carry out the examination is Hematology Reagent (Lyse, Diluent, Cleanser, Cellpack).

Data analysis. The research is an observational analytical study with a *cross sectional design*. The research samples were male and female patients, aged > 18 years, selected based on the results of examinations of patients from June to August 2021 who were declared to be suffering from COVID-19 based on positive results of the SARS-CoV-2 rT-PCR examination. Recording the results of laboratory examinations of routine hematology blood parameters from the Clinical Pathology Laboratory Installation on PDW Platelet Indice values, MPV. The number of samples used was 209 samples collected based on the severity of the patient recorded in the hospital medical record, namely mild category (1), moderate category (2) and severe category (3). PDW Platelet Indice value data, MPV and data on the severity of COVID-19 infection, were analyzed using SPSS 23 software (SPSS IBM License, USA). The data was tested using statistical analysis, the Annova test (Kruskal Wallis) followed by the Mann Whitney test to identify different groups.

RESULTS AND DISCUSSION

Characteristics of COVID-19 patients. Based on the analysis of the results, the average age of the 209 sufferers was 49.5 years (range 19 – 95 years), the ratio was 98 male sufferers (46.9%) versus 111 female sufferers (53.1%). Patient demographic data is presented in Table 1 as follows:

Table 1. Demographic characteristics of Covid-19 sufferers (n = 209)

Characteristics	Age (years)	amount
Age	Minimum	19
	Maximum	95
	Mean	49.5
Gender	Man	98 (46.9 %)
	Woman	111 (53.1 %)

The characteristics of the symptoms and signs experienced by Covid-19 sufferers for each category of mild, moderate and severe severity, in this study, are presented in Table 2 as follow:

Table 2. Clinical characteristics (symptoms and signs) of Covid-19 sufferers

Symptoms and signs	Degree of severity		
	Mild	Moderate	Severe
Fever	51 (56.7 %)	47 (67.1 %)	27 (55.1 %)
Cough	50 (55.6 %)	48 (68.6 %)	32 (65.3 %)
Throat pain	40 (44.4 %)	38 (54.3 %)	20 (40.8 %)
Nasal congestion	37 (41.1 %)	31(44.3 %)	16 (32.7 %)
Headache	18 (20.0 %)	22 (31.4 %)	29 (59.2 %)
Anosmia	27 (30.0 %)	30 (42.9 %)	26 (53.1 %)
Hard to breathe	19 (21.1 %)	21(30.0 %)	15 (30.6 %)
Diarrhea	15 (16.7 %)	8 (11.4 %)	6 (12.2 %)
Tachypnea	17 (18.9 %)	15 (21.4 %)	28 (57.1 %)
Pneumonia	7 (7.8 %)	43 (61.4 %)	35 (71.4 %)
Loss of consciousness	0	1 (1.4 %)	6 (12.2 %)
SPO ₂ ≤ 93 %	0	3 (4.3 %)	27 (55.1 %)
ARDS	0	1 (1.4 %)	8 (16.3 %)

Fever, cough and sore throat are the most common symptoms and signs experienced in mild, moderate and severe degrees. Especially in terms of severity, the symptoms most frequently experienced by COVID-19 sufferers were pneumonia at 71.4% and there was a decrease in consciousness at 12.2%, SPO₂ ≤ 93% at 55.1% and 16.3% experienced symptoms of ARDS. In a study by Yuki *et al.*, 2020, fever, cough, shortness of breath, and sore throat are mild symptoms. Even though these signs and symptoms are mild, if the patient has a poor prognosis, it can develop into severe pneumonia, pulmonary edema, ARDS, multiorgan failure, and death (Yuki *et al.*, 2020).

Analysis of the relationship between MPV platelet indice and severity. Based on the analysis of the average ranking (mean rank), MPV levels increase according to increasing risk severity. The results of statistical tests using Kruskal-Wallis showed that there was no significant difference in the average MPV levels based on the severity of Covid-19 patients (p=0.300). These results are presented in Table 3 as follow:

Table 3. Distribution of average MPV rating values, severity level of Covid-19 sufferers

Variable	N	Mean Rank	Kruskal-Wallis	p value
Severity				
Mild	90	98.59	2.41	0.3 00
Moderate	70	106.16		
Severe	49	115.11		
Total	209			

Analysis of the relationship between the PDW indice and the degree of severity. Based on the research results, the average rank (Mean Rank) of PDW levels increases according to increasing risk severity. The results of statistical tests using Kruskal-Wallis showed that there was a significant difference in average PDW levels based on the severity of Covid-19 patients ($p=0.004$). This is shown in Table 4 as follow:

Table 4. Distribution of average PDW level ranking values based on the severity of COVID-19 sufferers

Variable	N	Mean Rank	Kruskal-Wallis	p value
Severity				
Mild	90	91.99	11,201	0.004
Moderate	70	105.79		
Severe	49	127.78		
Total	209			

To find out the different groups, then proceed with the Mann Whitney test. The test results are presented in Table 5 as follows:

Table 5. Differences in average PDW levels based on the severity of COVID-19 sufferers

Degree of Severity	pValue	Conclusion
Mild vs Moderate	0.157	No different
Mild vs Severe	0.001	different
Moderate vs Severe	0.055	No different

The results showed that there was a difference in PDW indices levels in the mild degree to the severe degree of severity in Covid-19 sufferers. This is supported by several studies that the PDW indice reflects variations in platelet size, the PDW indice increases when platelet destruction increases and there are variations in the size of newly formed immature platelets (Gao, 2014). Increased cytokine release and inflammation lead to higher platelet production and increased platelet destruction. SARS-CoV-2 utilizes the Spike protein to enter host cells by binding to the angiotensin converting enzyme 2 (ACE2) on the host cell membrane. The transmembrane protease serine2, a serine protease, proteolytically cleaves and activates the *Spike protein* to facilitate SARS-CoV-2 virus cell membrane fusion. *Spike protein* potentiates *thrombus formation*, then coagulation factors are released, inflammatory cytokines are secreted so that platelet leukocyte aggregates are formed (Zhang *et al.*, 2020). These results are also in accordance with research by Guclu *et al.* (2020) which explained that the MPV and PDW platelet indice were found to increase at the initial arrival and follow-up examination on day 3 in patients who died. This is associated with changes in platelet counts and indices, possibly due to bone marrow infection, destruction of platelets by the immune system and consumption of platelets due to aggregation in the lungs. Increased numbers of young platelets are also functionally more active than older platelets so these changes may explain the increased platelet MPV and PDW indice (Guclu *et al.*, 2020). Bone marrow tissue also expresses a small number of ACE2 receptors on haematopoietic cell membranes so that the virus can infect cells and cause cell death (Lei *et al.*, 2020).

The PDW indice is a marker of thrombocytosis anisocytosis, which describes the size distribution of platelets produced by megakaryocytes and increases upon platelet activation, reflecting variations in platelet size (Osselaer *et al.*, 1997). The PDW indice increases when platelet destruction increases and there are variations in the size of newly formed immature platelets (Gao *et al.*, 2014). Increased cytokine release and inflammation lead to higher platelet production and increased platelet destruction. SARS-CoV-2 utilizes the Spike protein to enter host cells by binding to the angiotensin converting enzyme 2 (ACE2) on the host cell membrane. Serine transmembrane protease 2, a serine protease, proteolytically cleaves and activates the Spike protein to facilitate SARS-CoV-2 virus cell membrane fusion. Spike protein potentiates thrombus formation. Coagulation factors are released,

inflammatory cytokines are secreted and platelet leukocyte aggregates are formed (Zhang *et al.*, 2020).

In line with other research conducted by Guner *et al* (2021), the MPV indice is not a good predictor for the severity of COVID-19 disease, the study concluded that there was no significant difference in the MPV indice between groups of children with confirmed and suspected COVID-19. Patients with confirmed COVID-19 had significantly lower platelet counts (Guner *et al.*, 2021). In patients with severe conditions, there were no significant differences in PLT, MPV, PCT between mild, moderate and severe groups (Lin *et al.*, 2021). As with the study by Huang *et al* (2020), there were no significant differences in hemoglobin, *Red Blood Cell Distribution Width* (RDW), MPV, PCT, or *Platelet* (PLT) levels between patients with mild and severe disease at admission, although RDW and MPV are indicators of inflammation (Huang *et al.*, 2020). In a study by Tian *et al* (2020), the PLT, PCT and MPV parameters were within the reference/normal value range in all cases. The average MPV value decreased sharply after day 7 and even approached the lower limit (Tian *et al.*, 2020). Changes in MPV levels are not only influenced by abnormalities in platelet counts, but are also related to the laboratory analysis methods used (Vasudeva *et al.*, 2019). Many factors including race, age, smoking, alcohol consumption, and physical activity can modify MPV (Budak *et al.*, 2016). Like other respiratory Viruses, SARS-CoV-2 infection implies distinct hematological changes, which help in the evaluation and monitoring of disease severity. The most common changes in the hematopoietic system are lymphocytopenia, neutrophilia, thrombocytosis or thrombocytopenia (Debuc *et al.*, 2020).

The platelet index is a marker that has the potential to be a diagnostic marker for various diseases, but its usefulness is limited by certain limitations. Preanalytical factors, such as *Ethylene Diamine Tetraacetic Acid* (EDTA), blood sample temperature or the time between blood sampling and testing can influence platelet index measurements, low sample temperature causes platelet volume to decrease, while heating causes the MPV indice to increase (Korniluk *et al.*, 2019). For example, EDTA causes platelets to shift from a discoid shape to a round shape resulting in variations in MPV values (Park *et al.*, 2002). Extended sample storage time causes a decrease in the PDW indice (Vagdatli *et al.*, 2010). In addition, different methods used (optics, impedance) and analyzer calibration affect the measurement results, indicating the urgent need for standardization of platelet indice measurements (Hong *et al.*, 2009).

CONCLUSION

Based on the results of the test analysis, there was a significant difference in the average PDW levels for COVID-19 sufferers ($p=0.004$), so this shows that the PDW platelet indice can be a predictor of the severity of COVID-19 sufferers.

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