

The Trend of Platelet-To-Lymphocyte Ratio (PLR) and Absolute-Lymphocyte-Count (ALC) in the Severity of Covid-19 Patients

Markus Gatot Sura Wijaya ¹, Arie Utariani ^{2*}, Hamzah ³, Bambang Pujo Semedi ⁴ and Prananda Surya Airlangga ⁴

¹ Anesthesiologist; Anesthesiology and Intensive Therapy Department, Faculty of Medicine Universitas Airlangga Surabaya Indonesia.

² Doctorate & Consultant of Pediatric Anesthesia; Anesthesiology and Intensive Therapy Department, Faculty of Medicine Universitas Airlangga Surabaya.

³ Doctorate & Consultant of Neuro Anesthesia; Anesthesiology and Intensive Therapy Department, Faculty of Medicine Universitas Airlangga Surabaya.

⁴ Consultant of Intensive Care; Anesthesiology and Intensive Therapy Department, Faculty of Medicine Universitas Airlangga Surabaya.

E-mail: xuz1997@126.com; 17853465307@163.com; 15969671713@163.com; 1141614547@qq.com; 13295351580@163.com; 1149094331@qq.com

*Corresponding author details: Arie Utariani; arieutariani1955@gmail.com

ABSTRACT

Background: Cytokine storms and severe immune injury are suspected as the cause of the occurrence of Acute Respiratory Distress Syndrome (ARDS), sepsis and various another organ failure. The trend of Platelet-to-Lymphocyte Ratio (PLR) and Absolute Lymphocyte Count (ALC) were expected to show the inflammatory process and the cytokine storm in the severity of Covid-19 patients. This research's purpose is to analyze the time course and dynamic changes of PLR and ALC in each severity.

Method: The research method used was an observational analytic study with a prospective cohort design. The research sample taken was Covid-19 patients admitted from July to October 2020 who met the inclusion and exclusion criteria. PLR, ALC was counted from the CBC (Complete Blood Count) examination checked every three days. The PLR and ALC trend of 14-days were then analyzed.

Result: A total of 119 research subjects were involved in this study. PLR is significantly different at day-0 and the day-3 with p-value <0.001 and 0.003. On the 3rd day of treatment, PLR of moderate severity rose rapidly. Mild, severe, and critical cases' PLR rose until day-6. After the day-6, PLR of all severity class tend to decrease. ALC was significantly different on day-0, day-3, day-6 and day-9 with p-value <0.001, 0.006, 0.024 and 0.001. ALC day-0 in critical patients is the lowest, and tend to be the lowest throughout the 14 days. ALC in mild, moderate and severe declined until day 6, then began to rise until day 14.

Conclusion: In Conclusion, PLR tend to increase from day-0 of treatment and peak in day-3 or day-6 of treatment and then decreased gradually until day-14 of the treatment. Meanwhile, ALC tend to decrease from day-0 of treatment and reach the lowest point at day-6 of treatment and then increased gradually until day-14 of the treatment. PLR was significantly different among the severity in day-0 and day-3 with p-value <0.001 and 0.003. ALC was significantly different among the severity in day-0, day-3, day-6 and day-9 with p-value <0.001, 0.006, 0.024 and 0.001.

Keywords: Platelet to Lymphocyte Ratio (PLR); Absolute Lymphocyte Count (ALC); Covid-19; Dynamic changes; Complete Blood Count (CBC)

INTRODUCTION

The first case of Covid-19 was reported in Wuhan in early December 2019 [1] Covid-19 spread rapidly from one city to the rest of the country in just 30 days. Case Fatality Rate (CFR) total of 2.3%, in patients over 80 years old 14.8%, in patients aged 70-79 years 8.0%, and 49.0% in patients who arrive at the critical stage [2]. Cytokine storms and severe immune injury are suspected as the cause of the occurrence of ARDS, sepsis and various other organ failure [3].

Platelet serves as a target and also as an effector that strengthens inflammatory signals [4]. Lymphocytes are the effectors of the immune system. After activating, naïve T lymphocytes will proliferate in accordance with the antigen presented by APC, activated B lymphocytes will undergo proliferation and differentiation to produce appropriate antibodies [5].

A complete blood test is a simple, inexpensive and routinely performed examination that can measure Absolute Lymphocyte Count (ALC) and Platelet-to-Lymphocyte Ratio (PLR). The trend of PLR and ALC were expected to show the inflammatory process and the cytokine storm in the severity of Covid-19 patients. This research's purpose is to analyze the time course and dynamic changes of PLR and ALC in each severity.

METHOD

The research method used was an observational analytic study with a prospective cohort design. The ethical clearance was granted by Komite Etik Penelitian Kesehatan Dr. Soetomo General Hospital Surabaya (0009/KEPK/VI/2020) in Juni 4th 2020. The research population was Covid-19 patients in Dr. Soetomo General Hospital. The research sample taken was Covid-19 patients admitted dr. Soetomo General Hospital Surabaya, from July until October 2020, met the inclusion and exclusion criteria.

Inclusion criteria were;

- 1) Covid-19 confirmed cases,
- 2) suspected cases,
- 3) Age is more than 18 years old
- 4) The patient or the family or relatives agreed to sign the informed consent.

Exclusion criteria were;

- 1) patient died before the blood sampling
- 2) Patient with history of immunodeficiency diseases
- 3) patient with a history of autoimmune diseases,
- 4) Patient with pregnancy,
- 5) patient with chronic liver disease and chronic kidney disease
- 6) patient with neoplasm,
- 7) patient with active Tuberculosis Infection
- 8) patient taking immunosuppressant drugs.

Patients are dropped out from the study if;

- 1) never confirmed positive on RT-PCR examinations,
- 2) Patient rejecting the treatment, asking to drop out from the study, or asking for hospital discharge.

This study began by examining the history of the patient's disease course, comorbidities, SOFA Score, and referral system of the patient. PLR, ALC was counted from the CBC (Complete Blood Count) examination, which is being checked every three days as per the Clinical Practice Guidelines. The trend of 14-days subjects' PLR and ALC was then analyzed.

Patients were being categorized as their severity. Mild case is defined as mild symptoms and there are no manifestations of viral pneumonia or signs of hypoxia. Moderate case is defined as patients have symptoms of pneumonia such as fever, cough, shortness of breath, and rapid breathing, but there are no signs of severe pneumonia, SpO₂ is still >90% with room air. Severe case is defined as patient has symptoms of pneumonia such as fever, cough, shortness of breath, and rapid breathing, in the presence of one of the following signs; respiratory rate > 30x/m, severe breathing distress, or SpO₂ < 90% with room air. Critical case is defined as patients with ARDS and/or Sepsis or Septic Shock [6].

Patients also undergo treatment in accordance with the applicable Clinical Practice Guidelines for Covid-19 of the RSUD Dr. Soetomo. Patients were followed up until they died or were discharged.

Statistical analysis is conducted using Statistical Package for the Social Sciences (SPSS) 25th version. The normality of the distribution is analyzed by Kolmogorov-Smirnov

test. The trend of the PLR and ALC will be describe with linear chart. PLR and ALC difference on each severity is analyzed with Kruskal-Wallis test if not-normally distributed, or with ANOVA test if normally distributed.

RESULT

A total of 142 patients were eligible as the inclusion and exclusion criteria of this study. A total of 19 patients was dropped out because they never confirmed positive on PCR examinations, and four patients were dropped out because the patients rejected the treatment and asked for hospital discharge.

TABLE 1: Demographic and Characteristic of the Subjects

Variable N= 119	Median (IQR) N (%)
Age (Year)	52 (43-58)
Weight (kg)	70.0 (60-80)
BMI	25.78 (23.4-29.14)
Time of Onset to Admission (days)	7 (5-10)
Initial SOFA Score	3 (2-5)
Initial Blood Sugar (mg/dl)	147 (113-235)
LOS (days)	15 (9-22)
Sex	
Male	78 (65.5%)
Female	41 (34.5%)
Arrival	
Referral	73 (61.3%)
Without Referral	46 (38.7%)
Comorbidity	
DM	48 (40.3%)
Hypertension	34 (28.6%)
Cardiac Problem	8 (6.7%)
Lung Problem	6 (5.0%)
Overweight	46 (38.6%)
Obesitas	22 (18.5%)
Severity	
Mild	29 (24.4%)
Moderate	19 (16.0%)
Severe	18 (15.1%)
Critical	53 (44.5%)
Wards	
ICU	57 (47.9%)
Non-ICU	62 (52.1%)

Notes; BMI: Body Mass Index; LOS: Length of Stay; DM: Diabetes Melitus; ICU: Intensive Care Unit; Cardiac Problem includes Old Myocardial Infarction, Heart Failure, Arrhythmia. Lung Problem includes asthma and chronic obstruction pulmonary disease (COPD).

A total of 119 research subjects were involved in this study. The median age was 52 years with an interquartile range (IQR) of 43-58. The median weight was 70 kg (60-80). The median BMI was 25.78 (23.4-29.14). The median time of onset to admission was 7 (5-10). Median initial SOFA score was 3 (2-5). The median of initial blood sugar was 147 (113-235) mg/dl. The median length of stay was 15 (9-22) days. By gender, 78 (65.5%) were male, and 41 (34.5%) were female. Total 73 (61.3%) patients came to Dr Soetomo General Hospital through referral and 46 (38.7%) the patient without referral. Comorbid in the sample of patients was DM 48 people (40.3%), hypertension 34 people (28.6%), heart disease 37 people (31.1%), overweight 46 people (38.6%) lung disease, and obesity 22 people (18.5%). The severity of the incident was mild 29 cases (24.4%), moderate 19 cases (16.0%), severe 18 cases (15.1%), and critical 53 cases (44.5%). A total of 57 patients (47.9%) were treated in the ICU, and 62 patients were (52.1%) treated in ICU wards.

TABLE 2: Trend of PLR on each severity.

Severity	PLR Day-0 Median (IQR)	PLR Day-3 Median (IQR)	PLR Day-6 Median (IQR)	PLR Day-9 Median (IQR)	PLR Day-12 Median (IQR)	PLR Day-14 Median (IQR)
Mild	154.6 (111.9-194.75)	193.35 (146.64-299.77)	277.40 (141.50-373.00)	209.30 (135.00-312.50)	241.30 (186.55-300.90)	215.70 (172.95-291.75)
Moderate	234.30 (185.80-318.90)	373.05 (225.55-549.25)	390.90 (234.90-443.00)	343.80 (198.05-492.98)	264.70 (135.30-374.20)	210.80 (172.35-232.28)
Severe	209.75 (156.88-261.92)	318.40 (231.80-411.70)	336.40 (268.70-460.20)	312.10 (197.03-411.28)	247.85 (138.65-341.58)	189.35 (102.55-304.28)
Critical	269.00 (177.70-404.10)	287.10 (220.30-438.80)	341.30 (188.68-524.77)	296.00 (175.80-465.80)	223.30 (140.30-350.50)	201.30 (156.75-243.00)
p-value *	<0.001	0.003	0.155	0.295	0.983	0.953

Notes;

*: Kruskal-Wallis test, significantly different if p-value <0.05.

PLR trend from the admission until the 14th day on mild, moderate, heavy, and critical severity is recorded in table 2. PLR is significantly different at day-0 and the day-3 with p-value <0.001 and 0.003. PLR trend description can be seen in Figure 1. At the beginning of admission, PLR of critical patients were the highest with a median value of

269.00 (177.70-404.10), followed by moderate patients 234.30 (185.80-318.90), Severe 209.75 (156.88-261.92), and mild 154.6 (111.9-194.75). However, on the 3rd day of treatment, PLR moderate cases rose rapidly to 373.05 (225.55-549.25). After the 6th day, PLR at all severity levels drops.

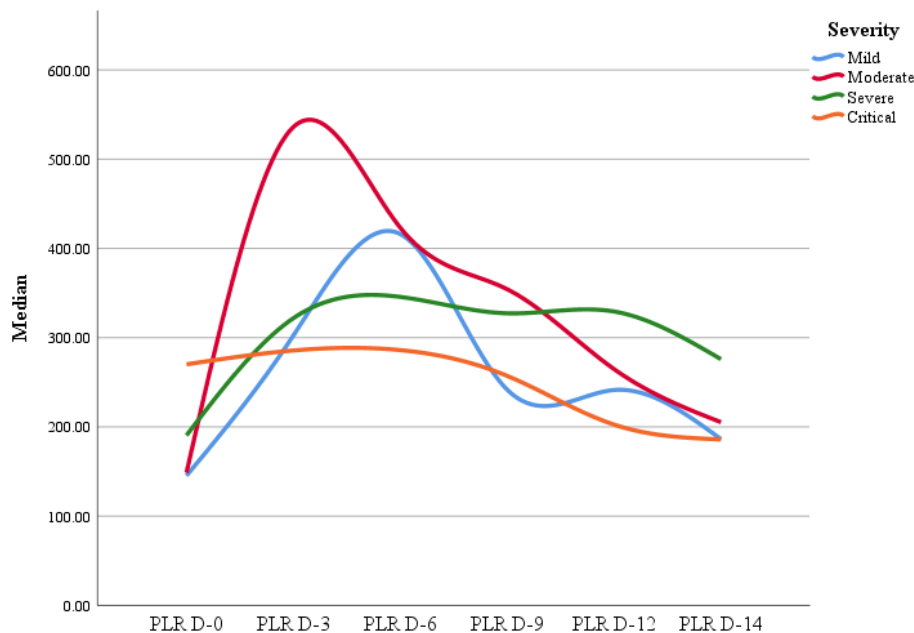


FIGURE 1: Trend of PLR on each Severity

TABLE 3: Trend of ALC on each severity

Severity	ALC Day-0 Median (IQR)	ALC Day-3 Median (IQR)	ALC Day-6 Median (IQR)	ALC Day-9 Median (IQR)	ALC Day-12 Median (IQR)	ALC Day-14 Median (IQR)
Mild	1.52 (1.21-2.05)	1.43 (1.04-2.02)	1.41 (1.10-1.93)	2.06 (1.33-2.67)	1.72 (1.57-2.13)	1.91 (1.38-2.41)
Moderate	1.15 (0.95-1.43)	1.11 (0.73-1.29)	1.29 (0.96-1.70)	1.46 (0.94-2.15)	2.13 (1.12-2.59)	1.60 (1.19-2.40)
Severe	1.42 (0.95-1.77)	1.18 (0.70-1.47)	1.20 (0.73-1.81)	1.80 (0.92-2.13)	2.22 (1.38-2.99)	2.02 (1.59-2.30)
Critical	0.89 (0.60-1.24)	0.94 (0.61-1.43)	1.03 (0.64-1.50)	1.07 (0.79-1.55)	1.37 (0.80-1.94)	1.45 (0.85-1.88)
p-value *	<0.001	0.006	0.024	0.001	0.230	0.194

Notes;

*: Kruskal-Wallis test, significantly different if p-value <0.05.

The ALC trend up to day 14 on mild, moderate, heavy, and critical severity is recorded in table 3. ALC was significantly different on day-0, day-3, day-6 and day-9 with p-value <0.001, 0.006, 0.024 and 0.001. An overview of ALC trends can be seen in Figure 2.

ALC day-0 in critical patients is the lowest with a median of 0.89 (0.60-1.24) and tend to be the lowest throughout the 14 days of the treatment. ALC in mild, moderate and severe declined until day 6. After day 6, the ALC began to rise until day 14.

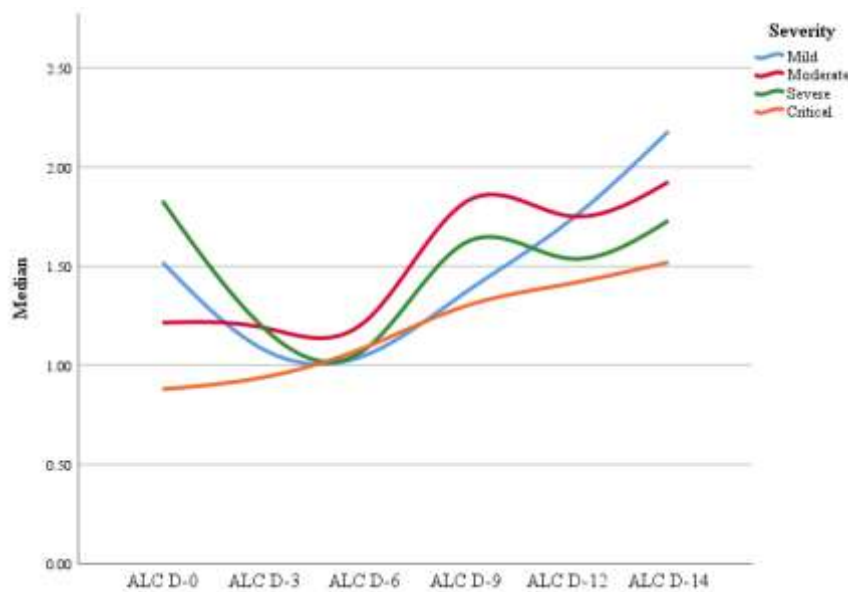


FIGURE 2: Trend of ALC on each Severity

DISCUSSION

The main causes of ARDS, sepsis and various other organ failures in Covid-19 are suspected to be cytokine storms and *severe immune injury*. Initial reports on Covid-19 in China mentioned an increase in pro-inflammatory cytokines IL1b, IFN γ , IP10, and MCP1, as well as anti-inflammatory cytokines IL4 and IL10 [7]. Cytokine storms are associated with an increase in pro-inflammatory cytokines in the body. Cytokines proinflammatory IL1b, IFN γ , IP10, and MCP1, cytokines antiinflammatory IL4 and IL10 were found to increase in Covid-19 [7]. Sixteen cytokines (M-CSF, IL-10, IFN- α 2, IL-13, IL-17, IL-4, IP-10, IL-1 β , IL-7, IL-1ra, G-CSF, IL-12, IFN- γ , IL-1 α , IL-2, HGF and PDGF-BB) correlated with *viral load* of Covid-19, and 14 cytokines (IL-12, IFN- γ , IL-2, HGF, IFN- α 2, IL-14, IL-17, IP-10, G-CSF, IL-10, IL-1ra, M-CSF, IL-1 α and IL-7) correlated with Murray Score to assess lung *injury* [8].

Platelets are both a target and an inflammatory booster. Lymphocytes are the effectors of inflammation and infection. Platelet activation will trigger platelet degranulation and release of chemokine related to recruitment, upregulation of lymphocyte function, as well as migration of lymphocytes to inflammatory tissues [4]. PLR is an approach of platelet and lymphocyte count ratio to describe the relationship of platelet and lymphocyte function in inflammation [9].

PLR at all severity indicates an increase from day-0 to day-3 or day-6. After that, PLR tends to go down. This increase in PLR can be caused by an increase in the number of platelets and / or a decrease in the number of lymphocytes. The increase in platelet count is due to its role as an inflammatory effector and relayer. PLR day-0 in patients with critical conditions is higher than others, but from its progress, the trend of plr critical patients tends to be flat. In contrast to mild, moderate and severe conditions where plr increases sharply until the 3rd day and then gradually decreases. Chen also noted similar results where the initial PLR on admission in critical patients was higher than other patients, and then decreased until the end of treatment. [10]

ALC day-0 or when the admission of critical patients is lowest compared to other conditions. Over time, ALC in critical patients tends to increase. But unlike in mild, moderate and severe cases, the ALC tends to drop until day 6, and then back up. ALC differs significantly on days 0, 3, 6, and 9. On day 12, the ALC of all four severities did not differ significantly. Chen also noted similar results that ALC day-0 in critical patients was lowest compared to others [10].

Research on the time course of chest CT describes the existence of four phases of lung picture in Covid-19 patients [11]. Stage one, early stage, 0-4 days from the beginning of symptoms. GGO (Ground Glass Opacity) is what can be found at this stage. Stage two, progressive stage, 5-8 days of onset of symptoms. The pulmonary picture shows a wider GGO, bilateral multilobes GGO, crazy paving pattern, and consolidation. Stage Three, Peak Stage, 9-13 days after symptoms, the picture of consolidation is more dominant. Stage four, Absorption stage, Infection is successfully controlled and the picture of consolidation in the lungs begins to be absorbed.

The median time symptom onset to admission in this study was 7 days. It means admitted patients, on average are on stage two, progressive stage. The picture of PLR trend continues to increase until the 3rd-6th day of hospital treatment, or 10-13 days of initial symptoms, which indicates that the patient is in peak stage. After that, PLR tends to decrease, this is appropriate where in patients there is a stage four or absorption stage. ALC also tends to decrease until the 6th day or the 13th day of symptoms, peak stage, then tend to rise again on stage four or absorption stage.

CONCLUSION

In Conclusion, PLR tend to increase from day-0 of treatment and peak in day-3 or day-6 of treatment and then decreased gradually until day-14 of the treatment. Meanwhile, ALC tend to decrease from day-0 of treatment and reach the lowest point at day-6 of treatment and then increased gradually until day-14 of the treatment. PLR was significantly different among the severity in day-0 and day-

3 with p-value <0.001 and 0.003. ALC was significantly different among the severity in day-0, day-3, day-6 and day-9 with p-value <0.001, 0.006, 0.024 and 0.001. The Trend of PLR of the critical patients were higher at day-0 of the treatment, but it tends to be the lowest throughout the 14 days of the treatment. Meanwhile, ALC of the critical patients tend to be the lowest throughout the 14 days of the treatment.

REFERENCES

- [1] Guan W-J, Ni Z-Y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020; Available from: 10.1056/NEJMoa2002032
- [2] Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. *JAMA - J Am Med Assoc*. 2020; Available from: 10.1001/jama.2020.2648
- [3] Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020; Available from: 10.1016/S2213-2600(20)30076-X
- [4] Stokes KY, Granger DN. Platelets: A critical link between inflammation and microvascular dysfunction. *J Physiol*. 2012;590(5):1023–34. Available from: 10.1113/jphysiol.2011.225417
- [5] Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular immunology*. Ninth Edit. Philadelphia: Elsevier; 2018.
- [6] World Health Organization. *Clinical Management of Covid-19: Interim Guidance 27 May 2020* [Internet]. Geneva: World Health Organisation; 2020.
- [7] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497–506. Available from: 10.1016/S0140-6736(20)30183-5
- [8] Liu Y, Zhang C, Huang F, et al. Elevated plasma levels of selective cytokines in COVID-19 patients reflect viral load and lung injury. *Natl Sci Rev*. 2020;7(6):1003–11. Available from: 10.1093/nsr/nwaa037
- [9] Thomas M, Storey R. The role of platelets in inflammation. *Thromb Haemost*. 2015;114(09):449–58. Available from: 10.1160/TH14-12-1067
- [10] Chen R, Sang L, Jiang M, et al. Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. *J Allergy Clin Immunol*. 2020;146(1):89–100. Available from: 10.1016/j.jaci.2020.05.003
- [11] Pan F, Ye T, Sun P, et al. Time Course of Lung Changes at Chest CT during Recovery from Coronavirus Disease 2019 (COVID-19). *Radiology*. 2020;295(3):715–21. Available from: 10.1148/radiol.2020200370