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Interleukin-6 Level and Neutrophil-Lymphocytes Ratio and Severity of Coronavirus Disease 19

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Abstract

Interleukin-6 (IL-6) is a proinflammatory cytokine that plays an important role in hyperinflammation and cytokine storm in Coronavirus Disease 19 (COVID-19) patients. The neutrophil-lymphocyte ratio (NRL) describes the innate and adaptive immune responses. Elevated IL-6 and NLR levels usually indicate a severe clinical condition in COVID-19 patients. Aim of this study was to determine the correlation of IL-6 and NLR with the severity of illness in COVID-19 hospitalized patients. This was a descriptive correlative observational study with a cross-sectional design using secondary data from COVID-19 patients treated in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from November 2020 to October 2021. There were 225 subjects who were classified by the severity and analyzed for IL-6 levels and NLR. Median levels of IL-6 at moderate, severe, and critical levels were 4.1 pg/mL, 20.4 pg/mL, and 38.8 pg/mL, respectively. The median NLR at moderate, severe, and critical grades were respectively 4.41, 9.65, and 17.79. The correlation between IL-6, NLR, and severity was 0.441 ($p<0.001$) and 0.408 ($p<0.001$). Meanwhile, the correlation between IL-6 levels and NLR in COVID-19 was 0.230 ($p<0.001$). Thus, IL-6 and NRL levels have a moderate positive correlation with the severity of COVID-19, while IL-6 and NRL have a weak correlation because IL-6 is not the only factor that affects the NRL.

Keywords: COVID-19, disease severity, IL-6, NRL

Introduction

Coronavirus Disease 19 (COVID-19) is a disease caused by the Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The World Health Organization (WHO) officially declared COVID-19 as a pandemic in March 2020. The number of COVID-19 cases has reached 200 million people with 4 million deaths worldwide in July 2021.[1]

Acute Respiratory Syndrome Coronavirus-2 will infect respiratory epithelial cells through the ACE-2 receptor. Infected epithelial cells induce innate immune cells. Antigen presenting cells (APC) will present viral antigens to T helper cell/CD4+ lymphocytes, thereby activating these cells. COVID-19 infection will be limited to the lungs and manifests mildly or even without symptoms if the immune response is good, whereas if the immune response is poor, it can cause a cytokine storm, causing severe clinical manifestations that can develop into multiorgan failure (MOF), Acute respiratory distress syndrome (ARDS), and have a poor prognosis. Interleukin-6 is a proinflammatory cytokine that plays a major role in cytokine storm and clinical manifestations of COVID-19 patients.[2-4]

IL-6 examination is very necessary, especially for treatment guidelines, but not all health services have the examination tool. Interleukin-6 released by immune cells can cause dysregulation, fatigue, and apoptosis of lymphocyte cells, resulting in a decrease in lymphocyte cells. Interleukin-6 can increase the production and recruitment of neutrophils and prolong the life span of neutrophils. An increase in neutrophils and a decrease in lymphocytes causes an increase in NLR (Neutrophil lymphocyte ratio). The routine hematological examination is simple and can determine NLR which is one of the markers of inflammation besides IL-6. In

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moderate COVID-19 conditions, IL-6 and NLR levels will begin to increase, and IL-6 and NLR levels increase as the disease progresses. This study aims to examine the correlation of IL-6 and NLR with the severity of COVID-19 patients. [5,6]

Methods

This study used secondary data from subjects with confirmed COVID-19 who were hospitalized at RSUP Dr. Hasan Sadikin Bandung from November 2020 to October 31, 2021. This study received ethical approval from The Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung. The data taken are clinical data of research subjects from medical records and laboratory results from the Laboratory Information System (LIS). Hasan Sadikin Bandung. Data on the clinical stage of the patient was taken from the medical record section of Dr. RSUP. Hasan Sadikin Bandung. IL-6 examination and leukocyte count were taken through LIS at the Clinical Pathology section of Dr. RSUP. Hasan Sadikin Bandung. Sampling was based on purposive consecutive sampling. The inclusion criteria for research subjects were patients aged >18 years with confirmed COVID-19 who were examined for IL-6 and leukocyte count on the same day at the beginning of hospital admission with moderate, severe, and critical clinical manifestations. Exclusion criteria in this study were suffering from or having malignancy, autoimmune disease, HIV, aplastic anemia and receiving anti-inflammatory drugs and/or corticosteroids. The number of subjects included in this study was 230 subjects. A total of 3 subjects were excluded because they had a history of malignancy and 2 subjects excluded had a history of autoimmune at the time of recruitment of research subjects. The number of subjects who met the inclusion criteria and did not meet the exclusion criteria was 225 subjects.

The design of this study was a descriptive, correlative cross-sectional study using secondary data. Statistical analysis was carried out to determine the significant correlation between IL-6 levels and NLR values in COVID-19 patients with moderate, severe, and critical stages. The research data were analyzed using the Statistical Package for the Social Science (SPSS) program. The research data were tested for normality using the Kolmogorov Smirnov's test. The levels of IL-6 and NLR in this study had an abnormal distribution, so the Spearman Rank correlation test was carried out. The r coefficient of the Spearman Rank correlation test means that if you get a value of 0.00 – 0.09 then the correlation can be ignored, the value is 0.10 – 0.39 then the correlation is weak, the value is 0.40 – 0.69 then the correlation is moderate, the value is 0.70 –0.89 means a strong correlation, and a value of 0.90–1.00 means a very strong correlation. All of these tests used a 95% confidence level (α=0.05) and a p-value of <0.05 was considered statistically significant.

Results

The results of the normality test for age, leukocyte count, neutrophil count, lymphocyte count, and IL-6 levels were not normally distributed and presented in the form of the median and interquartile range (IQR). The data on the characteristics of the research subjects can be seen in Table 1.

The number of subjects in this study was 225 people, with a median age of 59 years, 63.6% male and 36.4% female. The most severity disease stage was severe (52.0%), and the most comorbid was hypertension (43.6%).

The correlation between IL-6 levels and the degree of severity in research subjects was tested using Spearman's rank test because the data for IL-6 levels were not normally distributed. The results of Spearman's rank test between IL-6 levels and the degree of disease severity in research subjects in tabular form can be seen in Table 2.

The results in Table 2 show that the median IL-6 at moderate was 4.1 pg/mL (IQR: 2.7–10.1 pg/mL), then at severe was 20.4 pg/mL (IQR: 8.6 –49.2 pg/mL) and critical was 38.8 pg/mL (IQR: 15.3–123.8 pg/mL).

The correlation between NLR levels and severity in research subjects was tested using Spearman's rank test because the data for NLR levels were not normally distributed. The results of Spearman's rank test between NLR and the degree of disease severity in research subjects can be seen in Table 3.

The results in Table 3 show that the median NLR at moderate was 4.41 (IQR: 3.59–11.17), severe was 9.65 (IQR: 5.39–18.39), and critical was 9.65. 17.79 (IQR: 9.47–25.27). The boxplot graph of the correlation between IL-6 and NLR with severity can be seen in Figure 1 below

In the bloxspot image above, there is a positive correlation with moderate strength between IL-6 and severity (r=0.441, p<0.001), and there is a positive correlation with moderates strength

Table 1 Subject Characteristics

Variable	Amount (n=225)
Age (years), median (IQR)	59 (49-67)
Gender, n (%)	
Man	143 (63.6)
Woman	82 (36.4)
Severity, n (%)	
Moderate	38 (16.9)
Severe	117 (52.0)
Critical	70 (31.1)
Comorbid disease, n (%)	
Diabetes mellitus	66 (29.3)
Hypertension	98 (43.6)
Obesity	19 (8.4)
Heart disease	63 (28.0)
Leukocyte Count/μL, median (IQR)	10.780 (6.850–15.675)
Neutrophil Count/μL, median (IQR)	8.600 (5.415–14.480)
Lymphocyte CountL, median (IQR)	820 (545–1.300)
NLR, median (IQR)	10.61 (5.26–20.21)
IL-6 (pg/mL), median (IQR)	20.40 (6.25–55.75)

Note: n=frequency, %=percentage, SD=*Standard Deviation*, IQR=*Interquartile Range*

Table 2 Correlation of IL-6 Levels with the Severity of COVID-19

Variable	Severity			r coefficient	p-value
	Moderate	Severe	Critical		
IL-6 Median (IQR) pg/mL	4.1 (2.7 – 10.1)	20.4 (8.6 – 49.2)	38.8 (15.3 – 123.8)	0.441	<0.001*

Note: IQR=Interquartile Range data analysis using Spearman's rank correlation test, r = Spearman correlation coefficient
*p<0.05=significant

Table 3 Correlation of NLR Levels with the Severity of COVID-19

Variable	Severity				p value
	Moderate	Severe	Critical	r coefficient	
NLR Median (IQR)	4.41 (3.59 – 11.17)	9.65 (5.39 – 18.39)	17.79 (9.47 – 25.27)	0.408	<0.001*

Note: IQR=Interquartile Range data analysis using Spearman's rank correlation test, r = Spearman correlation coefficient
*p<0.05=significant

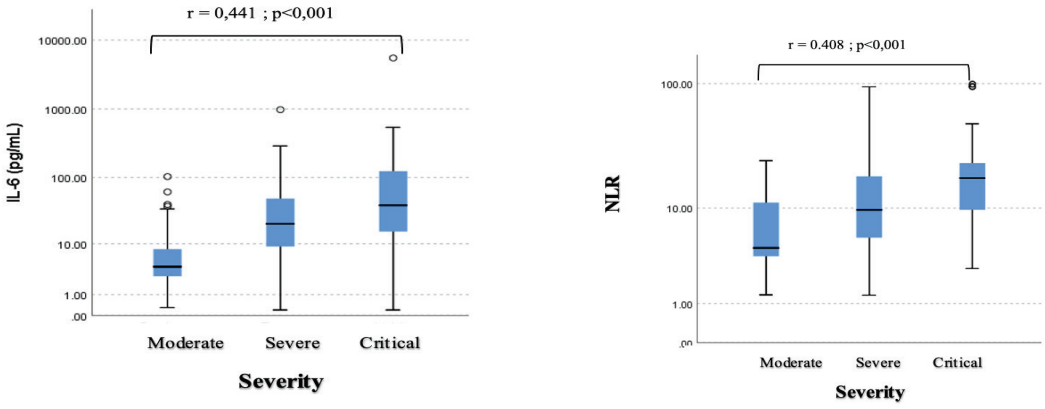

Figure 1 Boxplot Distribution of IL-6 and NLR by Severity of Disease

between NLR and severity in study subjects (r=0.408, p <0.001). The increase in IL-6 levels or NLR values, the more severe the severity of COVID-19.

The correlation between IL-6 levels and NLR in research subjects was tested by Spearman's rank test because IL-6 and NLR data not normally distributed. The results of the analysis using Spearman's rank correlation showed that there was a positive correlation with weak strength between IL-6 and NLR (r=0.230, p<0.001).

Discussion

Median age in this study was 59 years. Research Silva et al. in Brazil patients treated with moderate to severe degrees had a median age of 58.3 years. In this study, 166 subjects (71%) were older than 50 years. People over 50 years old tend to experience changes in function and cellular composition of innate and adaptive immunity (immunosenescence). Changes in T and B cell function and an increase in cytokine production causing people in that age tend to have more severe symptoms that they need hospitalized. At that age there is also an increased risk of developing comorbid diseases such as hypertension, diabetes mellitus, and cardiovascular disease.[7,8]

Researchers took the four most common comorbid diseases causing the risk of complications and death in COVID-19 based on previous research, namely hypertension, diabetes mellitus, cardiovascular and obesity. This study's most comorbid subjects were hypertension (43.6%). According to the study of Richardson et al.[9] in America and Zhou F et al.[10] in China, patients with hypertension were the most comorbid in COVID-19 patients. In hypertensive patients, an imbalance in the renin-angiotensin system can cause inflammation, thrombus, and other side effects that aggravate COVID-19 patients so that COVID-19 patients with hypertension tend to be treated. In addition, chronic hypertension can lead to decreased cardiovascular reserve, arterial wall stiffness, endothelial dysfunction, and left ventricular hypertrophy, making these patients susceptible to complications from COVID-19, such as myocardial injury, thrombosis, and acute kidney injury (AKI).[9,10]

The results of the examination of IL-6 levels in all study subjects had a median of 20.40 pg/mL. The researcher divided the subjects into three categories based on the severity of COVID-19 disease: moderate, severe, and critical. Subjects with a median grade of 4.1 pg/mL for IL-6 levels, subjects with severe degrees of IL-6 levels with a median of 20.4 pg/mL, and subjects with a critical grade with a median of 38.8 pg/mL. The results of the correlation analysis between IL-6 levels and severity showed a moderate positive correlation statistically (r=0.441, p<0.001). The correlation indicates that the more severe the severity, the higher the IL-6. The positive correlation of IL-6 with the severity of COVID-19 patients was similar to the meta-analysis study of Liu et al.[11] 2021 and Andriani et al.[12] in Indonesia. In the research of Liu et al., They found that the Spearman correlation has a value of r = 0.709 with p = 0.002 which means it has a strong correlation, while in this study, there is a moderate correlation. This study is because

there are differences in the research of Liu et al.[11] The characteristics of the study subjects were more varied than this study, only dividing the two groups between severe and not severe and not excluding the use of anti-inflammatory drugs and corticosteroids. In the research of Andriani et al[12], the Spearman correlation value r = 0.337 with p = 0.017 means it has a weak correlation compared to this study. The difference in this study is the number of subjects. The study from Andriani et al.[12] had 50 subjects, including subjects with mild degrees, in the analysis, and the use of anti-inflammatory and corticosteroids was not excluded. The progression of COVID-19 disease from severe to critical is thought to be strongly associated with a cytokine storm. Cytokine storms not only cause damage to epithelial and endothelial cells but also cause vascular leakage, which ultimately leads to ARDS and other severe conditions. In critically ill COVID-19 patients, there is abnormal production and regulation of proinflammatory cytokines. Proinflammatory cytokines that play a role in cytokine storm is IL-6. Interleukin-6 can induce other proinflammatory cytokines in immune cells, recruiting leukocytes and increasing migration from blood to organs, thereby aggravating the inflammatory state. Although there was a moderate correlation in this study, IL-6 is a proinflammatory cytokine that plays a crucial role during a cytokine storm. Other factors can influence disease development, such as other inflammatory cytokines (TNFα, IFN type 1, and IL-10), the condition of immune cells, and comorbidities in patients.[11-15]

The results of the correlation analysis between NLR and severity showed a moderate positive correlation which was statistically significant (r=0.408, p<0.001). The correlation shows that the more severe the severity, the higher the NLR value. This study followed the research of Suartono et al.[16] 2021 in Indonesia (r=564, p<0.001), which states that the NLR value has a moderate correlation to the severity of COVID-19 patients. The higher neutrophil-lymphocyte ratio is due to an increase in the number of neutrophils and a decrease in lymphocytes. The neutrophil-lymphocyte ratio reflects the balance between innate immunity (neutrophils) and adaptive immunity (lymphocytes). A higher NLR indicates that in severe cases, a dysregulated immune response indicates an overactive innate immune tendency in the immune system. This exaggerated inflammatory response can exacerbate the cytokine storm and increase tissue damage. Although neutrophils are part of innate immunity and lymphocytes are part of the adaptive immune system, many other components of the immune system influence the state of infection and the severity of COVID-19 patients. The components of innate immunity consist of epithelial cells, dendritic cells, natural killer cells, phagocytes, macrophages, and complement proteins. The main components of the adaptive immune system consist of lymphocytes and antibodies.[16-18]

The results of the correlation analysis between IL-6 and NLR levels showed a weak positive correlation which was statistically significant (r=0.230, p<0.001). In this study, there is a positive correlation following the research of Garcia-Gordillo et al.[19] in Mexico, Sayah et al.[20] in Algeria, and Liu et al.[17] in China. In the study of Garcia-Gordillo et al.[19] in Mexico (r = 0.485, p < 0.001) and Sayah et al.[20] in Algeria (r=0.634, p<0.001) which had a moderate correlation between IL-6 and NLR while this study it had a weak correlation, this may be due to differences in the inclusion and exclusion criteria. According to research by Liu et al.[17] in China (r=0.359, p<0.005), IL-6 had a weak correlation with NLR. Interleukin-6 is known to increase the production of maturation and activation of neutrophils and stimulate lymphocyte apoptosis to increase the value of NLR. Neutrophils are also one of the producers of IL-6, although not the main one. The correlation between IL-6 and NLR is weak because other processes influence the increase in neutrophils and the decrease in lymphocytes besides the increase in IL-6. The production and activation of neutrophils increased, also influenced by GM-CSF, IL-17, and Th17 cells and the presence of secondary infection. The reduction of lymphocytes caused by fatigue and lymphocyte apoptosis is not only affected by IL-6; other cytokines, such as IL-10 and TNF- can stimulate lymphocyte fatigue and apoptosis. SARS-CoV-2 that binds directly to the ACE-2 receptor on lymphocytes may also interfere with lymphocyte function directly. Lung damage caused by COVID-19 causes the accumulation of lactic acid in the body, which can interfere with lymphocyte function and cause a decrease in lymphocytes.[5,17,19,20]

The limitation of the study is that the researcher did not know the history of vaccination and the SARS-CoV-2 variant. The researcher took the study at one time without seeing any changes in the severity status of the study subjects during treatment.

This study concludes that IL-6 levels and NLR values had a moderately positive correlation with the severity of COVID-19 while the correlation

between IL-6 and NLR in COVID-19 patients is weak because IL-6 is not the only factor affecting NLR. Interleukin-6 and NLR examinations are expected to increase clinician awareness in managing COVID-19.

References

1. IRIS. COVID-19 weekly epidemiological update. 50 ed. Geneva: World Health Organization; 2021.
2. Bohn MK, Hall A, Sepiashvili L, Jung B, Steele S, Adeli K. Pathophysiology of COVID-19: mechanisms underlying disease severity and progression. Physiology (Bethesda). 2020;35(5):288–301. doi:10.1152/physiol.00019.2020
3. Burhan E, Susanto AD, Isbaniah F, Nasution SA, Ginanjar E, Pitoyo CW, et al, Editors. Pedoman Tatalaksana COVID-19. 4 ed. Jakarta: PDPI, PERKI, PAPDI, PERDATIN, IDAI; 2022.
4. Murphy K, Weaver C, Editors. Janeway's immunobiology. 9th ed. New york: Garland science; 2016.
5. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, et al. COVID-19: immunopathogenesis and Immunotherapeutics. Signal transduction and targeted therapy. 2020;5(1):1–8.
6. Yang P-H, Ding Y-B, Xu Z, Pu R, Li P, Yan J, et al. Increased circulating level of interleukin-6 and CD8+ T cell exhaustion are associated with progression of COVID-19. Infectious Diseases of Poverty. 2020;9(1):161. doi:10.1186/s40249-020-00780-6
7. Silva B, Rodrigues W, Abadia D, Alves da Silva D, Andrade E. Clinical-Epidemiology Aspect of Inpatients With Moderate or Severe COVID-19 in a Brazilian Macroregion: Disease and Countermeasures. Front Cell Infect Microbiol. 2022;12:899702. doi:10.3389/fcimb.2022.899702
8. Jameson JL, Kasper DL, Longo DL, Fauci AS, Hauser SL, Loscalzo J, Editors. Harrison's principles of internal medicine. 20th ed. United States: McGraw-Hill; 2018.
9. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. JAMA. 2020;323(20):2052–9. doi:10.1001/jama.2020.6775
10. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–62. doi:10.1016/s0140-6736(20)30566-3
11. Liu X, Wang H, Shi S, Xiao J. Association between IL-6 and severe disease and mortality in COVID-19 disease: a systematic review and meta-analysis. Postgraduate Medical Journal. 2021:postgradmedj-2021-139939. doi:10.1136/postgradmedj-2021-139939
12. Andriani I, Utariani A, Hanindito E, Airlangga PS. Correlation between CRP Il-6 level, severity and mortality in patients with Covid-19 infection in Indonesia. International J Scientific Advances. 2021;2(4):2708.
13. Hashizume M. Outlook of IL-6 signaling blockade for COVID-19 pneumonia. Inflammation and Regeneration. 2020;40(1):24. doi:10.1186/s41232-020-00134-7
14. Skevaki C, Fragkou PC, Cheng C, Xie M, Renz H. Laboratory characteristics of patients infected with the novel SARS-CoV-2 virus. J Infection. 2020;81(2):205–12. doi:https://doi.org/10.1016/j.jinf.2020.06.039
15. Henry BM, Oliveira MHSd, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020;58(7):1021–28. doi:doi:10.1515/cclm-2020-0369
16. Suhartono S, Wijaya I, Dalimoenthe NZ. The correlation of neutrophil-to-lymphocyte ratio (NLR) and monocytes-to-lymphocytes ratio (MLR) with disease severity in hospitalized patients with Coronavirus disease 2019 (COVID-19). Bali Med J. 2021;10(2):653–8.
17. Liu L, Zheng Y, Cai L, Wu W, Tang S, Ding Y, et al. Neutrophil-to-lymphocyte ratio, a critical predictor for assessment of disease severity in patients with COVID-19. International journal of laboratory hematology. 2021;43(2):329–35.
18. Lanier L. The innate and adaptive immune systems. In: Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P, Editors. Molecular biology of the cell. 6 ed. New York: Garland Science; 2015. p. 1297–39.
19. Garcia-Gordillo JA, Camiro-Zúñiga A, Aguilar-Soto M, Cuenca D, Cadena-Fernández A, Khouri LS, et al. COVID-IRS: A novel predictive score for risk of invasive mechanical ventilation in patients with COVID-19. PLOS

ONE. 2021;16(4):e0248357. doi:10.1371/journal.pone.0248357

20. Sayah W, Berkane I, Guermache I, Sabri M, Lakhal FZ, Yasmine Rahali S, et al. Interleukin-6, procalcitonin and neutrophil-to-lymphocyte ratio: Potential immune-inflammatory parameters to identify severe and fatal forms of COVID-19. Cytokine. 2021;141:155428. doi:10.1016/j.cyto.2021.155428