

CORRELATION ANALYSIS OF INTERLEUKIN-6 (IL-6) LEVEL AND NUMBER OF LYMPHOCYTES IN COVID-19 POSITIVE PATIENTS AT PERSAHABATAN HOSPITAL

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ABSTRACT

COVID-19 is caused by the SARS-CoV-2 virus and is transmitted through droplets. In COVID-19 patients who are critical or have severe symptoms, hypercytokines are often found, which is characterized by an increase in the cytokine Interleukin-6 (IL-6). High levels of IL-6 cytokines can disrupt the immune system, including leukocytes, especially lymphocytes, which are the main system of the immune response. The state of continued inflammation and continuously stimulated lymphocytes will cause the lymphocytes to work too hard. This study aims to determine the relationship of Interleukin-6 (IL-6) with lymphocytes in COVID-19 positive patients. This study is quantitative research with correlation analysis method using data collection technique by purposive sampling. Furthermore, to analyze, the authors used the Kolmogorov-Smirnov normality test and the Spearman correlation test. The results of the analysis showed a fairly significant correlation between levels of Interleukin-6 (IL-6) and the number of lymphocytes in Covid-19 positive patients.

Keywords: Covid-19, interleukin-6, hypercytokine, lymphocytopenia

ABSTRAK

COVID-19 disebabkan oleh virus SARS-COV-2 dan ditransmisi melalui droplet. Pada pasien-pasien COVID-19 yang kritis atau bergejala berat sering ditemukan terjadinya hipersitokin yang ditandai dengan meningkatnya sitokin Interleukin-6 (IL-6). Tingginya sitokin IL-6 ini dapat mengacaukan sistem imun, termasuk leukosit terutama limfosit yang merupakan sistem utama respons imun. Keadaan inflamasi yang terus berlanjut dan limfosit yang terus menerus distimulasi akan mengakibatkan limfosit bekerja terlalu berat. Penelitian ini bertujuan untuk mengetahui hubungan Interleukin-6 (IL-6) dengan limfosit pada pasien positif COVID-19. Penelitian ini adalah penelitian kuantitatif dengan metode analisis korelasi menggunakan teknik pengumpulan data secara *purposive sampling*. Selanjutnya untuk menganalisis, penulis menggunakan uji normalitas *Kolmogorov-Smirnov* dan uji korelasi *Spearman*. Hasil analisis menunjukkan korelasi yang cukup berhubungan antara kadar Interleukin-6 (IL-6) dengan jumlah limfosit pada pasien positif COVID-19.

Kata kunci: COVID-19, interleukin-6, hipersitokin, limfositopenia

INTRODUCTION

The SARS-CoV-2 virus is a new type of coronavirus that attacks humans by infecting the respiratory tract. This virus is transmitted through droplets from the respiratory tract or direct contact with droplets.¹ SARS-CoV-2 belongs to the *coronaviridae* family, with its genetic material being ss-RNA. Viruses consist of an *envelope* (E), *spike* (S), membrane (M), and *nucleocapsid* (N) protein coat. *Spike* is a glycoprotein with crown-like projections and plays an important role in pathogenesis with specific *host cell receptors* (Figure 1).^{2, 3}

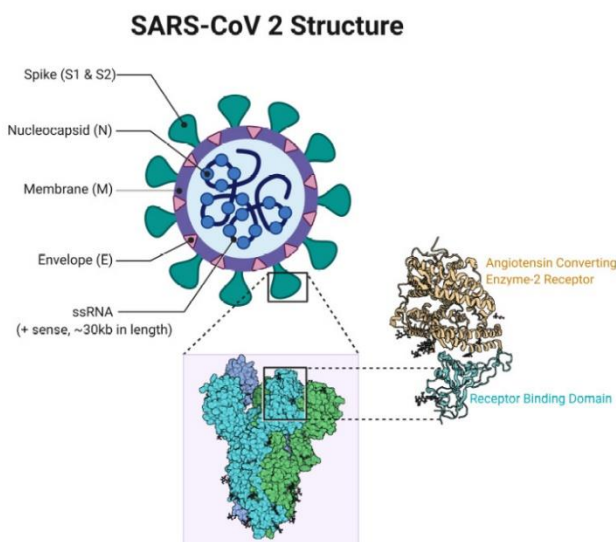


Figure 1. SARS-coV 2 Structure²⁴

Symptoms of COVID-19 are similar to pneumonia in general. The most common symptoms are fever and cough. Most patients also have shortness of breath. The diagnosis of COVID-19 can be confirmed by using a PCR (*Polymerase Chain Reaction*) test whose samples are taken from an upper respiratory tract swab (nasopharynx and

oropharynx) or by an antigen test (rapid test).⁴

At the end of 2019 in the city of Wuhan, Hubei Province, China, there were reports of cases of pneumonia with unknown etiology.⁵ The lung sample is then examined in this case. The results of the examination showed a new form of coronavirus. The International Committee on Taxonomy of Viruses later formally named this virus SARS-CoV-2. WHO then gave the name to the disease caused by this virus, namely COVID-19.⁶ The prevalence of COVID-19 has continued to increase from its initial discovery in Wuhan to eventually affecting more than 200 countries in the world. In March 2020 WHO declared COVID-19 a pandemic.⁷ Case reports in Indonesia based on the COVID-19 Task Force as of July 2020 recorded 70,736 confirmed cases, 37,651 recovered cases and 3,417 deaths.⁸

In COVID-19 patients with severe symptoms, the effects of the immune system's reaction to the virus can lead to *ARDS* (*Acute Respiratory Distress Syndrome*) and result in organ system failure.⁹ Hypercytokines are considered to be one of the main causes of *ARDS* and organ system failure.¹⁰ The cytokine used as a parameter for COVID-19 is the cytokine Interleukin-6 (IL-6).¹¹ Interleukin-6 (IL-6) is a proinflammatory cytokine produced by several types of cells, namely macrophages, dendritic cells, monocytes, mast cells,

fibroblasts, B lymphocytes, T lymphocytes and endothelial cells to stimulate the immune response during infection.¹² In the early stages of inflammation, IL-6 is produced by monocytes and macrophages.¹³ The occurrence of these hypercytokines disrupts the regulation of the immune system.¹¹

There are several factors that can explain the relationship between Sars-CoV-2, Interleukin-6 (IL-6) and the incidence of lymphopenia (reduced number of lymphocytes in the blood). Where in critical COVID-19 patients, high levels of IL-6 cytokines can disrupt the immune system, including leukocytes, especially lymphocytes, which are the main system of the immune response. The inflammatory state that continues and the lymphocytes are continuously stimulated will cause the lymphocytes to work too hard which then gradually decreases the performance of the lymphocytes and their numbers also decrease.¹⁴

High levels of IL-6 cytokines will also inhibit the process of lymphopoiesis (proliferation and maturation of B lymphocytes, T lymphocytes and NK cells in lymphoid organs) and cause apoptosis (programmed cell death) in lymphocytes.¹⁵ On the other hand, these cytokines induce and accelerate the processes of granulopoiesis (the process of formation and maturation of neutrophils, eosinophils and

basophils) and myelopoiesis (the process of formation and maturation of monocytes and bone marrow cells).¹⁶ Thus, this hypercytokine exerts an inverse correlation between the induction of granulopoiesis and lymphopoiesis in the bone marrow of SARS-CoV-2 patients. Lymphopoiesis is inhibited so that the production of lymphocytes is disrupted, causing the number of lymphocytes to decrease while myelopoiesis and granulopoiesis are stimulated so that the production of monocytes and neutrophils increases. As is known monocytes and neutrophils are one of the cells that produce IL-6.¹⁷ Many research on IL-6 and cytokines has been conducted elsewhere. Our study focus on IL-6 levels and its correlation analysis in COVID-19 patients. These two parameters play important role in patients recovery from COVID-19 diseases.^{14, 15}

METHODS

This research was conducted at Persahabatan Hospital in June – August 2021 using secondary data, namely medical records of patients who were confirmed positive for COVID-19. The research method is correlation analysis and *cross-sectional* research design. Ethical clearance were done in Persahabatan Hospital. Limitation on this research is our sample collection were only taken once. Thus there are no parallel comparable data from COVID-19 patient on IL-6 levels and blood

lymphocyte during COVID-19 disease.

IL-6 levels and lymphocyte levels were collected from blood samples. IL-6 levels were measured using Cobas e 411 analyzer. Reagent were taken out of the cooler and stored at room temperature for few minutes. First device were calibrated using calibrator and control. Blood samples were centrifuge at 6000 rpm. Afterwards, serum sample were injected and diluted (1:10) in the device.

Lymphocyte levels were measured using Sysmex XN-3000. Blood samples in K3EDTA vacutainer tubes were homogenized. After that barcode scan was pasted on these samples. Samples then arranged in the tray inside Sysmex XN-3000. Device automatically analyzed the samples and displayed the results on the monitor.

The sampling technique used is

purposive sampling, which is a sampling technique by selecting a sample among the population in accordance with what the researcher wants. The samples taken met the inclusion criteria, including patients who had been diagnosed as positive for COVID-19, had IL-6 and lymphocyte examinations done, patients were treated in intensive care unit (ICU) and patients that had comorbidities. From a total of 55 research populations obtained, 30 samples met the inclusion criteria for data processing.

RESULTS AND DISCUSSION

From a study conducted on 30 samples of positive COVID-19 patients who were hospitalized in the ICU of the Persahabatan Hospital, the results of Interleukin-6 (IL-6) and lymphocytes from June - July 2021 were listed in Table 1.

Tabel 1. COVID -19 Patients Hospitalized in the ICU of Persahabatan Hospital

NO	NAME	DIAGNOSIS	GENDER	AGE	IL-6 * ≤ 7 pg/mL	Lymphocytes *20-40%
1	Patient 1	HT	Woman	30	26.0	4
2	Patient 2	DM	Man	74	23.4	3
3	Patient 3	HD	Woman	39	34.8	3
4	Patient 4	HT	Man	38	38.2	10
5	Patient 5	DM	Man	53	52.1	8
6	Patient 6	DM	Man	72	88.7	6
7	Patient 7	HT	Man	35	22.6	8
8	Patient 8	HT	Woman	44	33.6	10
9	Patient 9	HT	Man	46	4.2	9
10	Patient 10	DM	Woman	33	72.2	7
11	Patient 11	KF	Man	50	81.6	4
12	Patient 12	DM	Woman	64	41.9	4
13	Patient 13	HD	Man	58	90.4	3
14	Patient 14	HT	Man	50	14.8	12

15	Patient 15	HD	Woman	50	7.4	27
16	Patient 16	DM	Man	51	15.2	13
17	patient 17	HD	Man	31	72.0	5
18	Patient 18	-	Woman	34	50.4	5
19	Patient 19	KF	Man	59	42.2	3
20	Patient 20	HD	Man	50	42.4	10
21	Patient 21	DM	Man	29	20.9	9
22	Patient 22	HT	Man	27	13.0	12
23	Patient 23	DM	Man	75	335.7	4
24	Patient 24	HT	Man	78	51.6	26
25	Patient 25	DM	Man	74	7.2	5
26	Patient 26	-	Woman	39	73.8	5
27	Patient 27	HD	Man	48	16.1	10
28	Patient 28	KF	Man	37	7.0	8
29	Patient 29	KF	Man	51	18.3	4
30	Patient 30	DM	Man	58	55.8	12

Description: * Normal Value; (DM= Diabetes Mellitus, KF= Kidney Failure, HT= Hypertension, HD=Heart disease)

The data in Table 1 shows that of the 30 samples, 28 samples or 93% of them had increased IL-6 results, and 28 samples or 93% had decreased lymphocytes.

Table 2. Normality Test Results

Variable	Sig	Information
IL-6	0.000	not normally distributed
Lymphocytes	0.010	not normally distributed

In Table 2, from the results of the normality test for IL-6, a significance value of 0.000 is obtained where the value is <0.05 so that the IL-6 data in this study is not normally distributed. The normality test for lymphocytes obtained a significance value of 0.010 where the value is <0.05 so that the lymphocyte data in this study was not normally distributed. Therefore statistical analysis used in this study is The Spearman Test.

Table 3. Results of Spearman Correlation Analysis

Variable Independent	Variable Dependent	Correlation Coefficient (r)	P-value (p)
IL-6	lymphocytes	- 0.378	0.040

Based on Table 3, the correlation between the levels of Interleukin-6 (IL-6) and the number of lymphocytes is - 0.378, which means that the correlation is sufficient. Where the criteria for the strength of the relationship (correlation coefficient) is 0.00 = no correlation; 0.00-0.25 = very weak correlation; 0.26-0.50 = sufficient correlation; 0.51-0.75 = strong correlation; 0.76-0.99 = very strong correlation; 1.00 = perfect correlation. The results of the analysis in Table 3 show that the correlation value on the correlation coefficient is negative, so the relationship between the two

variables is not unidirectional, thus the higher the level of Interleukin-6 (IL-6), the lower the number of lymphocytes. Results also displayed a significance value (p- value) of 0.040 which means that the two variables have a correlation. This is guided by the criteria for p-value <0.05, then it is correlated, while the p-value is >0.05, so it is not correlated.

Discussion

The results showed an increase from 30 patients with this severe condition, 28 patients (93%) had elevated IL-6 results (Table 1), this is as mentioned in a previous study in Wuhan.¹⁸ High IL-6 can disrupt the immune system including leukocytes, especially lymphocytes and cause the performance of lymphocytes to decrease so that the number also decreases, according to the results of lymphocytes obtained in this study where in 28 patients (93%) there was lymphopenia (reduced number of lymphocytes in the body). blood). This result is supported by research conducted by Li Tan in 2020.¹⁷

Based on the correlation analysis test in Table 3, which correlates IL-6 with lymphocytes, the results obtained are sufficient correlation. This result is different from previous research at Tongji Hospital in that there is a strong correlation.¹⁹ This may be due to differences in age categories in the research sample, where in this study the

largest sample was aged 60-68 years, while in this study, the largest sample was aged 36-55 years.²⁰

From the results of the study and the description above, it was found that there was a fairly correlated result. There was no strong relationship between Interleukin-6 (IL-6) and the number of lymphocytes. These results indicate that the high level of IL-6 in COVID-19 positive patients with severe symptoms will disrupt the immune system, one of which affects the performance of lymphocytes, causing their production of lymphocytes to decrease. The occurrence of an increase in Interleukin-6 (IL-6) is influenced by age and comorbidities. In patients with this condition the immune system is inadequate.

The SARS-CoV-2 was received via respiratory aerosols binding in the nasals epithelial cells of the respiratory tract. It binds to host receptor ACE-2 in the epithelial cells. The virus undergoes local replication and propagation, along with the infection of ciliated cells. At this stage the infected cells will release interferon. When interferon fail to overcome viral infection the virus undergo replications and produce more nucleocapsid. These lead immune system to produce many cytokines and inflammatory markers such as IL-1, IL-6, IL-8, IL-120 and IL-12), tumour

necrosis factor- α (TNF- α), IFN- λ and IFN- β , CXCL-10, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 α (MIP-1 α). This condition known as cytokine storm.²¹

Cytokine storm causes chemoattractant to neutrophils and some of lymphocytes, CD4 helper T cells and CD8 Cytotoxic T Cells. Lung cells then undergo apoptosis releasing new viral particles. Due to the persistent injury caused by the sequestered inflammatory cells and viral replication leading to loss of pneumocytes. This results in diffuse alveolar damage eventually culminating in an acute respiratory distress syndrome. (21) (22) Therefore analysis on IL-6 level correlations on Lymphocytes number gives important information understanding COVID-19 diseases. Other research in line with this research was in 2021. (23) They reported no correlation between IL-6 level and lymphocytes number. The Pearson correlation was conducted among the two groups and showed no correlation between IL-6 and lymphocyte level. Their research also showed IL-6 level and lymphocyte level may be responsible to the severity of the disease.²³

CONCLUSIONS AND SUGGESTIONS

Conclusion

Patients with bad conditions for COVID-19 are more common in patients

with comorbidities (comorbidities). In COVID-19 patients with severe symptoms, increased levels of IL-6 and decreased number of lymphocytes were found. There is a relationship between levels of Interleukin-6 (IL-6) and the number of lymphocytes in patients with severe symptoms of COVID-19.

Suggestion

Further research is expected to be able to analyze more deeply on lymphocytes, namely lymphocytes which are more specific, what lymphocyte clusters have an effect on the high IL-6 level. Related parties who wish to conduct similar research should add hemostasis parameters (blood clotting factors) such as D-dimer, Prothrombin Time (*Prothrombin Time*) and APTT (*Activated Partial Thromboplastin Time*).

The authors reported no potential conflict of interest on this research.

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