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Research Article

# Anti-Inflammatory Cytokine (IL-10) Profiles and Ratio of IL-6/IL-10 in Covid-19 Patients

**Shinta Dewi Permata Sari[1*], Erlin Listiyaningsih[2], Wening Tri Mawanti[3,5], Dewi Martalena[4,5]**

[1]Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, Indonesia
[2]Department of Public Health, Faculty of Medicine, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, Indonesia
[3]Department of Occupational Health, Faculty of Medicine, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, Indonesia
[4]Department of Internal Medicine, Faculty of Medicine, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, Indonesia
[5]Pondok Kopi Jakarta Islamic Hospital, Jakarta, Indonesia

*Corresponding author: shinta.dps@uhamka.ac.id

## ABSTRACT

**Background:** The number of COVID-19 cases in Indonesia has continually increased since the first cases appeared in March 2020. This disease is due to SARS-CoV-2 virus infection in the respiratory system that induces an immune response. The innate and adaptive immune response triggered the secretion of an excessive pro-inflammatory cytokine-caused cytokine storm that became one of the mechanisms of acute respiratory distress (ARDS). The anti-inflammatory cytokines (IL-10, IL-13, and IL-4) were secreted as the immune response in the ARDS condition. **Purposes:** This study aims to determine the ratio of the IL-6/IL-10 profile as basic information for the therapeutic approach to prevent ARDS. **Methods:** This cross-sectional study used stored biological material in plasma form from COVID-19 patients in Jakarta Islamic Hospital – Pondok Kopi and Dr. M. Goenawan Partowidigdo Hospital, Cisarua. The plasmas were from severe (n=20) patients and mild to moderate severity (n=25). The negative control sample was collected from 13 healthy subjects. Assessment of IL-10 levels in plasma using ELISA technique. **Results:** Our analysis showed that IL-10 has no statistical difference between negative control, mild to moderate, and severe categories (p=0.629). Meanwhile, the ratio IL-6/IL-10 presented statistical differences between mild to moderate and severe categories (p=0.011). The average ratio of IL-6/IL-10 in severe categories is two-fold higher than in mild-moderate categories. **Conclusion:** We conclude that there is a cytokine storm condition in severe COVID-19 patients with an imbalance ratio of pro-and anti-inflammatory cytokines and could be used as basic information for drug development in cytokine storm conditions to prevent ARDS.

**Keywords:** covid-19, cytokine, immune response, inflammation

## INTRODUCTION

Corona Virus Disease-19 (COVID-19) is an infectious disease in the human respiratory system caused by the SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus-2) virus / known as novel coronavirus. SARS-CoV-2 is a single-stranded RNA virus belonging to a beta-corona family member and has genetic similarities with SARS-CoV and MERS (1).

These viruses are transmitted from human to human and spread mainly from respiratory droplets of infected persons in confined places to the oral cavity and mucosal epithelial cells in the upper respiratory tract (2). The clinical symptoms of SARS-CoV-2 infection, such as fever, dry cough, fatigue, anosmia, and shortness of breath, appear within two days - 14 days after exposure (3). COVID-19 cases can be classified into four levels of severity: asymptomatic, mild, moderate, and severe. The virus enters the nasal system by exhalation of respiratory droplets from an infected individual or direct contact with a contaminated object. The virus will propagate in the nasal system, increasing viral load and inducing an innate immune response.

Furthermore, the virus migrates to the respiratory tract, and clinical symptoms such as fever, cough, nausea, and other acute upper respiratory symptoms appear. This condition is known as the mild category. Along with the mild and moderate phases, an innate and adaptive immune response will induce the elimination of the virus and prevent worsening illness. An impaired immune response to the virus may worsen the condition (4).

Migration of the SARS-CoV-2 virus from the upper respiratory to the lower respiratory system may induce the progression of severity. The presence of Angiotensin-Converting Enzyme 2 (ACE2) as the receptor for the SARS-CoV-2 virus in some system organs will result in the binding between the virus and ACE2 (5). This binding causes infiltration into the cell target, destruction of the cell, and clinical symptoms according to the organ target. This condition triggers an adaptive immune response to recruiting macrophages, monocytes, and T cells in response to infection. Suppose the immune response cannot resolve this infection. In that case, the immune cell (macrophage and T cell) will secrete an excessive pro-inflammatory cytokine (TNF-a, IL-6, IL-1, IL-8), causing ARDS and multi-organ failure (6). Some studies showed that elevated IL-6 levels are related to the severity of COVID-19 and increased risk of death (7–9). Age and various comorbid conditions, such as hypertension, asthma, diabetes, vascular disease, and cancer, could role as risk factors in the progression of COVID-19 (10,11).

Pro-inflammatory cytokine or viral infection induced another immune response to release an anti-inflammatory cytokine (IL-4, IL-10, TGF-b) by macrophages and T cells. The anti-inflammatory cytokines suppress inflammation and neoplastic processes by activating specific antigen T cells and inhibiting IFN-γ production. However, severe complications could occur in many infections due to excessive immune activation (12). Interleukin-10 (IL-10) is one of the anti-inflammatory cytokine systems that regulate and suppress the expression of pro-inflammatory cytokines during the infection recovery phase and consequently mitigate the damage caused by pro-inflammatory cytokines (13). The dynamics of pro-and anti-inflammatory cytokine levels may affect the degree of inflammation (14).

The dynamics in the immunological response to COVID-19 could be characterized by two cytokines, IL-6 and IL-10, that move the balance of patients from non-severe to severe, and hence evaluation of both markers is required to determine the line of diagnosis (15). Measuring the IL-6/IL-10 ratio will be crucial in identifying individuals more likely to undergo the progression of severity and taking the required precautions. Therefore, this study aims to result in a pro-and anti-inflammatory cytokine profile in COVID-19 patients with different severity that can be used to determine the specific treatment and as basic information for a therapeutic approach to prevent the ARDS and worsening condition.

## METHODS

### Study Design

This study design was an observational-analytical study, using samples of stored biological material in the form of plasma samples from COVID-19 patients. The samples were from COVID-19 patients in Islamic Pondok Kopi Hospital and DR. Goenawan Partowidigdo Hospital. The whole blood samples were collected in the previous study, and separated plasma was kept as stored biological material. In the last study, blood samples were collected on the first day of hospitalization or during the period of hospital treatment. The plasma sample was separated on the same day as the collected blood sample.

The plasma samples were from mild to moderate (n=25) and severe (n=20) patients. The negative control sample was from healthy subjects (n=13). The severity of patients was assessed on the first day of hospitalization or classified during the period of hospital treatment following the patient's current condition. The severity classification is as follows: Mild severity was a patient confirmed COVID-19 with upper respiratory system infection symptoms such as cough, fever, nausea, and fatigue, with or without anosmia. In moderate severity, the patient confirmed COVID-19 with clinical symptoms of pneumonia (fever, cough, shortness of breath, quick breath) and used nasal oxygen as breathing support. The severe severity was the patient confirmed COVID-19 with a lower respiratory infection with shortness of breath, pneumonia profile, and using a ventilator as breathing support.

The inclusion criteria were adult-geriatric COVID-19 hospitalized patients who consented to the blood collecting. The blood samples were collected on the first day of hospitalization, and the plasma sample was separated on the same day as the collected blood sample. The exclusion criteria were people who confirmed COVID-19 with PCR tests and were not hospitalized, pediatric COVID-19 hospitalized patients, and an adult–geriatric COVID-19 hospitalized patient who did not consent to the blood sampling. The Ethics Committee University of Muhammadiyah Prof. DR. HAMKA approved this study (KEPKK /FK/001/08/2020).

### Assessment of IL-10 in Plasma

The ELISA technique measured IL-10 in the plasma samples, based on literature measuring the anti-inflammatory cytokines in plasma (13,14,16). The measurement procedure followed the ELISA Kit insert package manual (Fine Test™/® ELISA kit).

### Statistical Analysis

Study results were analyzed statistically by IBM SPSS-22 software. The Kolmogorov-Smirnov test assessed the normality data results. The IL-10 data results were compared among severity groups and analyzed using the Kruskal-Wallis test. The IL-6/IL-10 ratio among groups was analyzed using a one-way ANOVA test. The comparison between mild to moderate severity was analyzed using an independent t-test and a Tukey test. Values of $p<0.05$ are shown as significant statistically.

## RESULTS

### IL-10 Levels

Figure 1 shows the distribution of IL-10 levels in the various levels of severity of COVID-19. The average IL-10 levels in the negative control, mild to moderate, and severe groups were 116.625 pg/mL, 108.325 pg/mL, and 87.313 pg/mL. The IL-10 levels were not distributed normally by the Kolmogorov-Smirnov normality test. This study results show that the difference in IL-10 levels between severity groups was not statistically significant (p= 0.579) based on the Kruskal-Wallis test. The highest IL-10 level in the mild to moderate group was 2-fold from the highest IL-10 level in the severe group. In addition, the average IL-10 levels in the severe group are lower than in the mild to moderate group and negative controls. Statistical analysis showed a negative correlation between the IL-10 levels and severity with the r count (-0.068) < r table (0.254). The IL-10 levels decreased insignificantly with increasing levels of severity. The decrease of IL-10 levels may be associated with elevated IL-6 levels, resulting in elevated pro-and anti-inflammatory cytokine ratios and a cytokine storm.

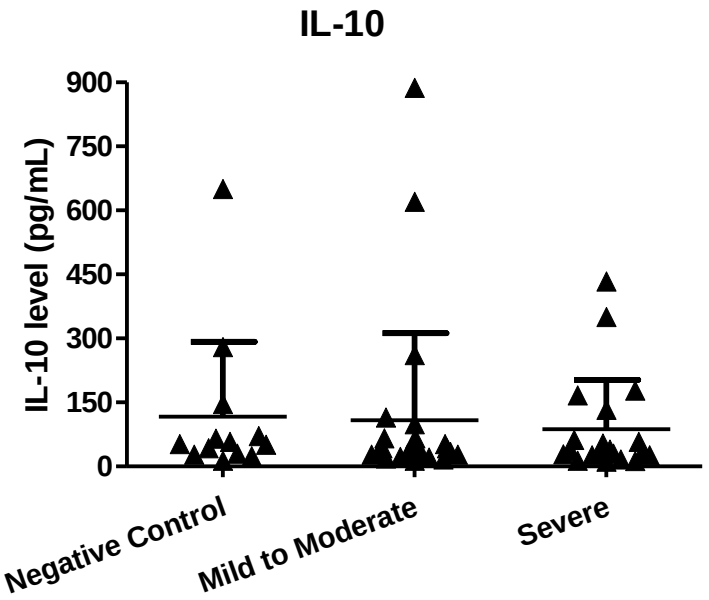


**Figure 1**. Distribution of the IL-10 levels in the negative control, mild to moderate, and severe categories.

### Ratio of IL-6/IL-10

The IL-6/IL-10 ratio profile could become one predictor of disease progression. The average IL-6/IL-10 ratio in the negative control, mild to moderate, and severe categories were 1.53, 1.63, and 3.4, respectively (Figure 2). Statistical analysis showed that the IL-6/IL-10 ratio was distributed normally. In addition, one-way ANOVA analysis showed significant differences in the IL-6/IL-10 ratio among groups (p=0.009). Meanwhile, there were no significant differences in the IL-6/IL-10 ratio between the negative control and the mild to moderate group. Moreover, this study showed a significant difference in the IL/6/IL-10 ratio between mild to moderate and severe categories ($p<0.05$) based on independent t-test analysis and the Tukey test. The high level of IL-10 caused the smallest ratio of IL-6/IL-10 in the negative control category. Another clinical condition of the subject can cause these results. In addition, some samples in the mild

to moderate category resulted in an IL-6/IL-10 ratio of more than one. It showed that the IL-6 levels in those samples were higher than the IL-10 levels.

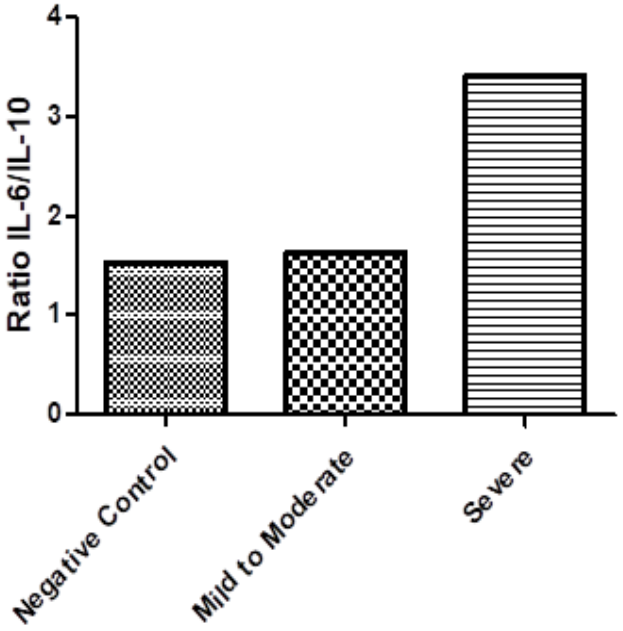


**Figure 2**. The ratio of IL-6/IL-10 in the negative control, mild to moderate, and severe categories.

Study results showed that 41 samples had an IL-6/IL-10 ratio higher than 1. In the mild to moderate group, 18 (72%) samples showed IL-6/IL-10 ratio > 1. Furthermore, 15 samples (75%) in the severe group presented IL-6/IL-10 ratio > 1. It shows that the IL-6 level and IL-10 level ratio are increased. The higher IL-6 levels than IL-10 levels could predict clinical outcomes in COVID-19 patients. Meanwhile, only one sample has an IL-6/IL-10 ratio close to 1. These findings indicate that those patients may have a lower risk of experiencing worsening conditions based on the balance of IL-6 and IL-10 levels.

## DISCUSSION

SARS-CoV-2 virus infection triggered an innate and adaptive immune response by its cytopathic nature. An innate immune response may cause disease in patients with asymptomatic and mild severity. The innate immune response is the primary guard in recognizing and fighting infection after viral entry (17). Moreover, an effective adaptive immune response could develop 2 – 3 weeks after virus exposure and be able to eliminate the virus. This immune response activation depends on the ability to recognize antigens by antibodies and or T-cells. In patients with severe conditions, the progression of an adaptive immune response may disrupt the first-line defense mechanisms. The virus-induced cell damage in infected tissues caused inflammation and secreted cytokines pro-inflammatory such as IL-6, IL-1b, TNF-a, and IL-1 (17). Another immune response secreted an anti-inflammatory cytokine in response to inflammation. IL-10 is an anti-inflammatory cytokine that suppresses Th-1 responses (18). The IL-10 function has many different and sometimes contradictory effects, such as suppressing and promoting inflammation and innate and adaptive immune responses.

This study shows that IL-10 levels are highly expressed in the mild to moderate group than in the severe group. The IL-10 exerts potent anti-inflammatory responsible for controlling the immune system's homeostatic balance. Impairment of IL-10 concentration may affect the immune response and severity of patients. A study by de Brito et al. showed the same result as ours, with the IL-10 levels in the severe group being lower than in the mild to moderate group (9). Merza et al.'s studies also showed that the IL-10 levels in the severe group were lower than in the moderate and control group (19). The IL-10 results in this study may be different from others' studies. Contrary to Han et al., Huang et al., and Zhao et al., studies showed elevated IL-10 levels in serum in severe patients (8,20,21). Lu et al. study also stated that IL-10 is a pro-inflammatory cytokine based on the serum levels in critical patients (22). These different results may show the contradictory function of IL-10 in COVID-19 patients.

Mortality in COVID-10 patients is caused by severe pneumonia and cellular damage mediated by proinflammatory mediators (23). Therefore, the IL-6 and IL-10 concentrations are frequently associated with the mortality of COVID-19 patients. The IL-10 is secreted during prolonged infection to maintain the homeostasis of the immune response. Considering the dynamics of pro-and anti-inflammatory cytokines in the role of an immune response, this study showed the elevated IL-6/IL-10 ratio in severe patients. Azaiz et al.'s study also presented an elevated IL6/IL-10 ratio in critical COVID-19 patients. These results could be one of the reasons for the occurrence of cytokine storms in ARDS conditions because of the insufficient level of anti-inflammatory cytokines in suppressing the proinflammatory cytokines, thus becoming one of the triggers for the deterioration of the COVID-19 patient's condition.

Data from several studies have revealed the balance between hyperinflammatory response and anti-inflammatory response role in the course of the disease (20,21,24). Depending on the IL-6/IL-10 profiling, severe COVID-19 patients will respond to blocking IL-6 alone or to both blocking of IL-6 and IL-10 or to blocking IL-10 alone. In the mild to moderate group, 72% of the sample presented an IL-6/IL-10 ratio > 1. These results show an elevated pro-and anti-inflammatory cytokines ratio. The IL-6 levels were increased with no enhancement of IL-10 levels. This increase could trigger the progression of the disease to severe conditions. Previous studies from Sari et al. showed the highest average levels of IL-6 among groups were in a severe category (7). The IL-6/IL-10 ratio could be a marker of the progression severity of COVID-19 patients. Moreover, IL-10 can be a potential targeted therapy in COVID-19 patients, especially in cytokine storm conditions, to decrease mortality. The pro- and anti-inflammatory data results contributed as supporting data for drug development in cytokine storm conditions. This study's limitation was the lack of other pro- and anti-inflammatory cytokines data and biochemical data parameters to compare with the severity. In addition, factors such as age, nutritional status, and comorbidities or physiological conditions also contributed to increasing cytokine production and need to be considered. This study defines that the ratio of IL-6/IL-10 in the severe group was abnormal compared to the negative control group from healthy subjects. To produce a comprehensive result, further research should be conducted using clinical laboratory parameters, including complete blood examination and biochemical parameters.

## CONCLUSION

The mild to moderate category had greater IL-10 levels than the severe category, but no statistically significant difference existed between the groups. The IL-6/IL-10 ratio in the severe categories was two-fold higher than in the mild to moderate categories. The ratio of pro-to-anti-inflammatory cytokines increased, which may be utilized as fundamental information for treatment approaches to avoid ARDS and worsening conditions.

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## CONFLICT OF INTEREST

This study has no conflict of interest.

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