**RESEARCH ARTICLE**

# Correlation of Interleukin-6 Level with Neutrophil to Lymphocyte Ratio and Disease Severity in COVID-19 Patients

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**Abstract**

Coronavirus disease 2019 (COVID-19) causes severe acute respiratory disease in humans and has spread rapidly worldwide since its first identification in December 2019. The neutrophil-to-lymphocyte ratio (NLR) describes the balance between the severity of inflammation and the immune system to be used as an important systemic inflammatory marker. Rapid progression of clinical deterioration is characterized by severe respiratory symptoms related to high levels of pro-inflammatory cytokines, like interleukin-6 (IL-6), indicating that the occurrence of cytokine storms leads to increased mortality. This study aims to assess the correlation between IL-6 and NLR in predicting the severity of COVID-19. This prospective cohort study was conducted at the COVID-19 ward of Universitas Sebelas Maret Hospital in August–September 2021. This study involved 66 COVID-19 patients >18 years old with asymptomatic to critical degree and Charlson Comorbidity Index (CCI) value ≤3. Examination of laboratory parameters and serum IL-6 was carried out when the patient entered the Emergency Room. Statistical test with Pearson's correlation test, significant if p<0.05. There is no significant correlation between IL-6 and NLR with p=0.56 and r=0.08, and a strong correlation between IL-6 and disease severity with p=0.000 and r=0.454. The conclusion is that IL-6 does not correlate with NLR and strongly correlates with disease severity in COVID-19 patients.

**Keywords:** COVID-19, disease severity, interleukin-6, neutrophil-to-lymphocyte ratio

## Introduction

The coronavirus disease (COVID-19) pandemic has caused a world health crisis. Most countries in the world are experiencing the COVID-19 pandemic.[1] The outbreak of infectious diseases poses a severe threat to global health. Asymptomatic, mild, and moderate symptoms are most symptoms experienced by COVID-19 patients. About 10–20% of cases experience severe symptoms, characterized by the fast development of acute respiratory distress syndrome (ARDS), sepsis, and multiple organ failure.[2–4] COVID-2019 pathogenesis is not fully understood, and the basic mechanisms of clinical severity remain unclear. Recently, immune dysfunction that triggers a cytokine storm has been associated with rapid disease progression. The researchers found that the severity of infection associated with biomarkers played an essential role in COVID-19 severity.[2]

The neutrophil-to-lymphocyte ratio (NLR) is a simple and readily available biomarker that can be calculated by the division between absolute neutrophil count and absolute lymphocyte count; it is a widely used laboratory marker for evaluating infectious diseases. The NLR value is a beneficial marker for assessing the severity and predicting the prognosis of pneumonia patients.[5,6] The previous study has shown that lower lymphocyte counts mainly decreased levels of T lymphocyte subsets, followed by an increase in pro-inflammatory cytokines, such as interleukin-6, associated with severe cases. This suggests that immune dysfunction and cytokine dysregulation are critical factors in the disease progression.[2]

There is no specific examination as a severity predictor for patients, so it is necessary to find factors that can be used to estimate clinical severity in COVID-19 patients, which will signal when the patients meet the predictors of death so they will get the maximum treatment immediately.[4,7] Our research aims to investigate the immune-inflammatory markers in the peripheral blood of patients with various forms of COVID-19, then evaluate the correlation between serum interleukin-6 and NLR in predicting disease severity in COVID-19 patients.

## Methods

This study was conducted at Universitas Sebelas Maret Hospital from August to September 2021. Inclusion criteria used in this research were COVID-19 patients >18 years old, confirmed using the reverse transcription-polymerase chain reaction (RT-PCR) method from a nasopharyngeal swab, and having Charlson Comorbidity Index (CCI) value ≤3 and patients who agreed to participate with signed informed consent.

Research subjects were classified according to the severity of symptoms based on the World Health Organization COVID-19 guidelines into asymptomatic, mild, moderate, severe, and critical cases. This study collects data regarding demographic characteristics, comorbidities, presenting symptoms, vital signs, initial laboratory parameters, and serum interleukin-6 (IL-6). NLR value is obtained from neutrophils divided by lymphocytes using a hematology analyzer—the examination of serum IL-6 using the ELISA method.

Our study is a prospective cohort study on 66 patients with COVID-19. The sampling technique was consecutive sampling—statistics analysis using Pearson correlation. The result is significant if p<0.05, and there is a strong correlation if r=0.4–0.6. All statistical analyses using SPSS 25.0 for Windows. This study has been approved by the local institutional review board, Universitas Sebelas Maret Health Research Ethics Committee, with ethical clearance number 90/UN27.06.6.1/KEP/EC/2021.

## Results

We analyzed 66 COVID-19 patients with all degrees of severity. Baseline characteristics, clinical and blood parameters of patients are shown in Table 1. The results of this study, numerical data (IL-6 value, NLR-value) were presented in mean±standard deviation. The total subject is 66 patients, 33 male and 33 female, divided into 29 patients in the asymptomatic group, 22 in the mild group, nine in the moderate group, four in the severe group, and two critical patients group. The critical group had the largest number of subjects. The critical group is accompanied by an increase in IL 6 corresponding to the patient's critical condition—demographic data are presented in Table 1.

According to the result of this study (Figure), the highest NLR value was found in the moderate group (6.70±4.50). In contrast, the highest IL-6 value was found in the critical group (121.33±27.14). Pearson's correlation analysis shows no correlation between IL-6 and NLR value (p=0.56).

There was a significant correlation between IL-6 and clinical severity, which is quite strong (r=0.45, p=0.00). Statistical analysis of the correlation between IL-6 with NLR and clinical severity can be seen in Table 2.

## Discussion

The lung is one of the main targets of SARS-CoV-2 infection; the virus binds to alveolar ACE-2 receptors, triggers the release of inflammatory factors, then activates the immune system, leading to a cytokine storm.[8,9] Acute systemic pathological inflammation is characteristic of COVID-19 infection with a high level of circulating pro-inflammatory cytokines.[10] Severe cases of COVID-19 have significantly decreased lymphocyte counts and increased neutrophil counts. Severe patients experience a significant increase in IL-6.[9] Precise and accurate identification of severe and critical cases is needed to provide appropriate management for high risks patients.[8] This study evaluated the correlation between IL-6 value and NLR and disease severity in COVID-19 patients.

This study showed that geriatric patients dominated the critical group. Some people may be more likely to have severe illnesses, including older age and certain underlying diseases.[11] Geng et al.[12] reported that with the increase in age, the severity of risk was gradually higher.

IL-6 value in the critical group is higher than in the other groups. COVID-19 patients with severe respiratory distress experience various immune dysregulation mediated by elevated IL-6. IL-6 is the strongest predictor of hypoxemic respiratory failure requiring oxygen therapy. A meta-analysis of 9 studies explains that elevated IL-6 was strongly correlated with severe cases. A study by Sabaka et al.[13] reported that severe COVID-19 patients had a mean IL-6 value of 58 pg/mL compared to 17 pg/mL in mild cases; IL-6 values >80 pg/mL predict the incidence of respiratory failure and require mechanical ventilation assistance in COVID-19. Another study by Galván-Román et al.,[14] showed that IL-6

**Table 1  Characteristics of Demographic and Clinical COVID-19 Patients**

| Variables | | Results |
|---|---|---|
| Age (years) (mean±SD) | Asymptomatic | 33.50±13.78 |
| | Mild | 43.18±17.28 |
| | Moderate | 55.11±6.57 |
| | Severe | 43.75±20.71 |
| | Critical | 74.50±14.85 |
| Gender | Male | 33 patients |
| | Female | 33 patients |
| IL-6 (pg/mL) (mean±SD) | Asymptomatic | 10.58±5.66 |
| | Mild | 19.37±16.63 |
| | Moderate | 40.33±50.20 |
| | Severe | 19.07±22.08 |
| | Critical | 121.33±27.14 |
| NLR (mean±SD) | Asymptomatic | 2.49±1.91 |
| | Mild | 5.10±4.62 |
| | Moderate | 6.70±4.50 |
| | Severe | 2.95±0.86 |
| | Critical | 4.20±4.07 |
| Outcome | | |
| Survive (mean±SD) | IL-6 (pg/ml) | 18.93±24.00 |
| | NLR (cells/uL) | 4.00±3.76 |
| | Male | 30 patients |
| | Female | 32 patients |
| Death (mean±SD) | IL-6 | 46.42±64.92 |
| | NLR (cells/uL) | 3.61±2.48 |
| | Male | 3 patients |
| | Female | 1 patient |
| Comorbid | Asymptomatic | DM (0 patient), HT (2 patients) |
| | Mild | DM (3 patients), HT (7 patients) |
| | Moderate | DM (2 patients), HT (4 patients) |
| | Severe | DM (1 patient), HT (0 patient) |
| | Critical | DM (1 patient), HT (1 patient) |

Note: DM: diabetes mellitus, HT: hypertension, SD: standard deviation
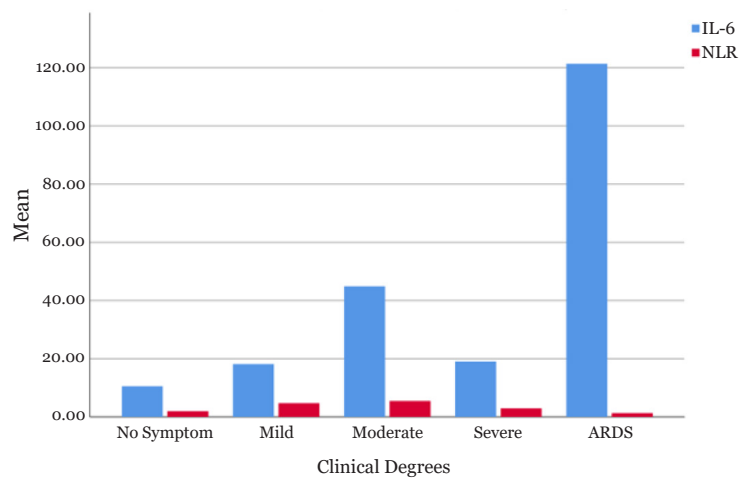


**Figure  Correlation between IL-6 and NLR to Clinical Severity**

### Table 1  Correlations between Serum IL-6 with NLR and Clinical Severity

| Variables | r | p |
|---|---|---|
| IL-6 with NLR | 0.08 | 0.56 |
| IL-6 with clinical severity | 0.45 | 0.00[*] |

Note: r: coefficient correlation; [*]Pearson's correlation, p<0.05 significant

level >30 pg/mL predict the need for invasive mechanical ventilation.

This study found the highest NLR value in moderate groups, 6.70±4.50, compared to 2.95±0.86 in the severe group and 4.20±4.07 in the critical group. NLR describes the compatibility between the degree of inflammation and the immune system and can be used as an important marker of systemic inflammatory response. A high NLR shows an imbalance in the inflammatory response and is a marker of disease severity.[15] NLR was positively associated with age, and geriatric had a higher value than the younger, and also slightly positively associated with systolic and diastolic pressure.[16] In this study, the moderate group has a higher NLR value than the critical group. Several possible reasons are that the moderate group had older age and more hypertension as comorbid than the critical group.

In this study, there was no correlation between IL-6 and NLR value. This result does not follow a study by Sayah et al.[17] that reported IL-6 positively correlated with NLR. When the number of neutrophils in the body is significantly reduced, the immune system is compromised and causes a significantly increased risk of infection. The number of lymphocytes in the body is associated with the immune system and the body's resistance to pathogenic microorganisms, and it has a negative correlation with the severity of inflammation.[18] Lymphocyte count below normal value associated with disease progression. SARS-CoV-2 may act on T lymphocytes, and T lymphocyte destruction is an important factor that causes deterioration of the patient's condition.[13] Based on this, we believe that severe COVID-19 infection causes significant systemic inflammation, characterized by elevated NLR, so that NLR can predict the severity of infection.[17] In this study, several reasons for no correlation between IL-6 and NLR are an equal distribution of comorbid factors and unequal population numbers in each group.

This study showed a significant correlation between IL-6 and clinical severity (r=0.45, p=0.00). IL-6 is a cytokine produced by macrophages that induces a pro-inflammatory response and is often elevated in COVID-19 patients.[19] IL-6 upregulation in COVID-19 lead to acute lung injury and acute systemic inflammatory response, which can be used as a predictor of an early marker of severe disease.[13,20] Study in Palembang, Indonesia, showed a correlation between IL-6 levels in COVID-19 with chest X-rays and clinical features.[21] A study by Guirao et al.[22] also showed that IL-6 could better predict COVID-19 disease severity. IL-6 level correlates with other clinical parameters to evaluate the severity of COVID-19. Overexpression of IL-6 is essential in increasing and spreading the cytokine storm leading to ARDS. The direct role of IL-6 in the pathogenesis of COVID-19 is further supported by the existence of the administration of therapy that inhibits IL-6 will improve the prognosis of severe COVID-19.[13]

### Conclusions

The clinical severity of COVID-19 is related to high peripheral blood immune-inflammatory markers. IL-6 and NLR values affect the clinical severity of COVID-19 patients and are important markers to evaluate the severe form and fatal development of COVID-19 in the context of hyperinflammation caused by cytokines. IL-6 can be an excellent marker to evaluate COVID-19 patients and provide appropriate treatment. However, NLR is a low-cost and accurate marker to predict clinical severity.

### Conflict of Interest

All authors state that there is no conflict of interest in this study.

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