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Interleukin-6 and Neutrophil–Lymphocyte Ratio in Predicting Outcome of Confirmed COVID-19 Patients

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ABSTRACT

Introduction: COVID-19 emerged as a pandemic about two years ago. Severe and critical COVID-19 has been associated with increased interleukin 6 (IL-6) levels and neutrophil–lymphocyte ratio (NLR). This study aimed to test whether IL-6 and/or NLR are associated with COVID-19 mortality.
Methods: Subjects were COVID-19 patients with suspected Omicron variant infection that were hospitalized at Dr. Moewardi General Hospital, Surakarta, from October 2021 to March 2022. According to their medical records, subjects were divided into survivor and non-survivor groups. At admission, serum levels of IL-6 and NLR were recorded, compared, and analyzed for association with mortality.
Results: Seventy-four respondents, average age of 53.07 ± 16.2 years old, joined the study. The area under curve (AUC) value of IL-6 was 0.740, with a cut-off value of 42.00 mg/dL (73.9% sensitivity; 70.6% specificity). The AUC value of NLR was 0.721, with a cut-off value of 5.51 (73.9% sensitivity; 60.8% specificity). IL-6 had a higher odds ratio than NLR as a risk factor for mortality (6.80 [95% CI 2.24–20.61; $p < 0.001$]; 4.39 [95% CI 1.48–13.03; $p < 0.001$], respectively). The correlation between IL-6 and NLR had an r-value of 0.164 ($p = 0.164$).
Conclusion: There was no difference in sensitivity between IL-6 level and NLR as mortality predictors of COVID-19, but serum IL-6 level was more specific. IL-6 level correlated positively with NLR, but there was no significance.

INTRODUCTION

Emerging in 2019, coronavirus infection is a novel disease which has become a global tragedy with a high mortality rate.[1] Manifestation of the disease can vary from asymptomatic to critical. Serious and critical clinical symptoms are more common in comorbidities such as heart failure, hypertension, diabetes mellitus, and stroke.[2]

Interleukin-6 (IL-6) plays a major role in the immune system by stimulating various host cells. In addition, IL-6 induces acute-phase proteins, megakaryocyte maturation and hematopoietic stem cell activation, differentiation of osteoclasts and angiogenesis in bone lesions, the proliferation of keratinocytes and mesangial cells, and persistence of myeloma and plasmacytoma.[3]

Acute respiratory distress syndrome (ARDS) with severe pneumonia, and sepsis with multiple end-organ failures caused by increased pro-inflammatory cytokines, are more common in severe and critically ill COVID-19 patients. In addition, elevated IL-6 levels are more common in ARDS.[4] Recent studies have recommended IL-6 screening in patient management to predict potentially worsening patients. However, this evaluation is relatively expensive and not available in

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healthcare facilities. Hence, a replacement marker is needed. Several other hematological parameters have been proposed as markers of the severity degree of COVID-19.[5] One or more of these could be useful as a replacement marker for IL-6 as a clinical parameter for patient care. Neutrophilia signifies an immunological response, while lymphopenia indicates "fatigue" of the immune system. Therefore, the ratio of neutrophils to lymphocytes can help predict severity and prognosis and avoid unnecessary examinations and treatments. In severe and critically ill patients, laboratory findings are characterized by lymphopenia and elevated levels of neutrophil–lymphocyte ratio (NLR) and IL-6. These three markers can be used as diagnostic and prognostic parameters.[4]

A previous study revealed that neutrophil and leukocyte counts were significantly greater with elevated than normal IL-6 levels, and lymphopenia was a severity or death risk factor in COVID-19 patients.[6] This study analyzed the biomarkers NLR and IL-6 as outcome predictors in COVID-19 patients to enable better and more effective treatments.

METHODS

Study Design and Subjects

The study design was a retrospective cohort that collected secondary data from patient medical records. Subjects were hospitalized patients with COVID-19, suspected of the Omicron variant, treated in the isolation ward of Dr. Moewardi General Hospital, Surakarta, from October 2021 to March 2022. The diagnosis of COVID-19 was confirmed by positive naso-oropharyngeal swab specimens on SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction (RT-PCR) testing. Inclusion criteria were patients (1) ≥18 years old, (2) with confirmed COVID-19 with positive RT-PCR, and (3) with IL-6 data. Exclusion criteria were (1) an autoimmune disorder from anamnesis or (2) a history of chronic pulmonary disease. Participants were then divided into survivor and non-survivor groups, and disease severity was noted. The degree of severity was classified as mild (respiratory rate 12–25 breaths/minute, oxygen saturation >95%), moderate (respiratory rate 20–30 breaths/minute, oxygen saturation <95%), severe (respiratory rate >30 breaths/minute, oxygen saturation <95%), and critical (any hemodynamic instability and/or respiratory failure).[1] The Health Research Ethics Committee, Dr. Moewardi General Hospital, Surakarta, approved this study protocol (No. 399/III/HREC/2022).

Laboratory Examination

Peripheral blood samples for laboratory examination were obtained from all patients' venous punctures during hospital admission. This study analyzed several parameters, including hemoglobin (Hb), leukocyte count, platelet count, differential count, NLR, and IL-6 levels. A routine hematology examination using the flow cytometry method was conducted using an XN-1000 automated hematology analyzer (Sysmex). IL-6 levels were examined using the automatic electrochemiluminescence (ECLIA) method on the COBAS® E411 (Roche Diagnostics) platform.

Statistical Analysis

The data are displayed as frequency distribution tables and diagrams. Statistical Package for the Social Science (SPSS) Statistics was used for all statistical tests with Windows 25th edition (IBM, USA). Data were not normally distributed, and logarithm transformation was subsequently applied. The Spearman correlation test was used to assess the correlation between IL-6 and NLR because the data remained abnormally distributed. Statistical significance was set at a p-value of <0.05.

This study evaluated the predictive value of various inflammatory biomarkers by taking the severity or mortality of COVID-19 as the dependent variable. This study used univariate and multivariate logistic regression analyses, and to determine the optimal predictive value and differentiate the limit value, this study used the receiver operator characteristic (ROC) curve. The odds ratio (OR) with a confidence interval of 95% (CI 95%) was estimated based on the cut-off value using the Fisher exact test.

RESULTS

Participant Characteristics

The total sample of this study was 74 COVID-19 patients who underwent treatment at Dr. Moewardi General Hospital, Surakarta. Table 1 summarizes the characteristics of the participants.

Table 1. Participant characteristics

Characteristic	Results (n = 74)
Age (years old)	53.07 ± 16.20
Sex	
Male	41 (55.4%)
Female	33 (44.6%)
Length of stay (days)	8.00 (3.00–19.00)
Severity	
Mild	14 (18.9%)
Moderate	37 (50.0%)
Severe	12 (16.2%)
Critical	11 (14.9%)
Comorbidities	
Diabetes	17 (23.0%)
Hypertension	26 (35.1%)
Cardiovascular	2 (2.7%)
Chronic lung disease	9 (12.2%)
Malignancy	9 (12.2%)
Liver disease	2 (2.7%)
Cerebrovascular disease	1 (1.4%)
Renal disease	1 (1.4%)
Pregnancy	1 (1.4%)
Fracture	7 (9.5%)
HIV	2 (2.7%)
Anemia	15 (20.3%)
Blood examination	
Hb (g/dL)	11.50 ± 2.19
Leucocyte count ($\times 10^3$/uL)	10.65 (1.20–35700.00)
Platelet count ($\times 10^3$/uL)	258.50 (50.00–372000.00)
Neutrophils (%)	78.80 (23.80–95.50)
Lymphocytes (%)	0.48 (0.01–136.76)
IL-6 (pg/mL)	33.00 (2.00–5000.00)
NLR	5.51 (0.13–50.26)

The average age was 53.07 ± 16.20 years old. There were more male (41, 55.4%) than female (33, 44.6%) patients. The median length of stay was 8.00 (3.00–19.00) days. The moderate category accounted for the greatest number of patients (37, 50.0%). Hypertension (26 patients, 35.1%) and anemia (15 patients, 20.3%) were the most common comorbidities.

The blood laboratory test results were as follows average hemoglobin level, 11.50 ± 2.19 (g/dL); median leukocyte count, 10.65 (1.20–35700.00) $\times 10^3$/uL; median platelet count, 258.50 (50.00–372,000.00) $\times 10^3$/uL; median neutrophil count, 78.80 (23.80–95.50) $\times 10^3$/uL; median lymphocyte count, 0.48 (0.01–136.76) $\times 10^3$/uL; median IL-6 level, 33.00 (2.00–5000.00) pg/mL; and median NLR, 5.51 (0.13–50.26).

Characteristics of Participants Based on Mortality

In this study, 55 patients were survivors, and 16 were non-survivors. An overview of the characteristics of the participants based on mortality is shown in Table 2.

There was no significant difference between survivor and non-survivor patients for age ($p = 0.411$), gender ($p = 0.378$), length of stay ($p = 0.805$), and comorbidities such as diabetes ($p = 0.305$), hypertension ($p = 0.313$), cardiovascular disease ($p = 0.528$), chronic lung disease ($p = 0.771$), malignancy ($p = 0.711$), liver disorders ($p = 0.528$), cerebrovascular disease ($p = 0.331$), kidney disease ($p = 1.000$), pregnancy ($p = 1.000$), and HIV ($p = 0.528$); and anemia ($p = 0.059$). There was a significant difference in severity ($p < 0.001$) between survivors and non-survivors, wherein patients who died tended to be severe (12.5%) or critical (87.5%).

Table 2. Characteristics of participants based on mortality

Characteristic	Survivors (n = 51)	Non-survivors (n = 23)	p
Age (years old)	52.02 ± 17.02	55.39 ± 14.29	0.411
Sex			0.378
Male	30 (58.8%)	11 (47.8%)	
Female	21 (41.2%)	12 (47.8%)	
Length of stay (days)	8.00 (3.00–19.00)	7.00 (4.00–16.00)	0.805
Severity			<0.001*
Mild	7 (12.7%)	0 (0.0%)	
Moderate	40 (72.7%)	0 (0.0%)	
Severe	4 (7.3%)	2 (12.5%)	
Critical	4 (7.3%)	14 (87.5%)	
Comorbidities			
Diabetes	10 (19.6%)	7 (30.4%)	0.305
Hypertension	16 (31.4%)	10 (43.5%)	0.313
Cardiovascular disease	1 (2.0%)	1 (4.3%)	0.528
Chronic lung disease	7 (13.7%)	2 (8.7%)	0.771
Malignancy	7 13.7%)	2 (8.7%)	0.771
Liver disease	1 (2.0%)	1 (4.3%)	0.528
Cerebrovascular disease	0 (0.0%)	1 (4.3%)	0.311
Renal disease	1 (2.0%)	0 (0.0%)	1.000
Pregnancy	1 (2.0%)	0 (0.0%)	1.000
Fracture	6 (11.8%)	1 (4.3%)	0.424
HIV	1 (2.0%)	1 (4.3%)	0.528
Anemia	7 (13.7%)	8 (34.8%)	0.059

Note: *Significant at $p < 0.05$

Differences in IL-6 and NLR Based on Mortality

The IL-6 and NLR examination data in this study were not normally distributed using the Kolmogorov–Smirnov test. The data were therefore displayed as median values (min–max) and then assessed with the Mann–Whitney test. The differences in IL-6 level and NLR based on the mortality of COVID-19 patients are shown in Table 3.

Table 3. Differences in IL-6 level and NLR based on the mortality

Variable	Survivors (n = 51)	Non-survivors (n = 23)	p
IL-6	23.00 (2.00–331.00)	70.00 (3.00–5000.00)	0.001#
NLR	4.61 (0.13–50.26)	8.01 (2.77–28.52)	0.002#

Note: #Significant at $p < 0.05$

IL-6 levels in the survivor group were found to have a median of 23.00 (2.00–331.00) pg/mL, whereas the non-survivor group had a higher median of 70.00 (3.00–5000.00) pg/mL. IL-6 levels were found to be statistically different between survivors and non-survivors.

The median NLR in survivors was 4.61 (0.13–50.26), while non-survivors had a higher median NLR of 8.01 (2.77–28.52). There was a statistically significant difference in the NLR values between survivor and non-survivor groups ($p = 0.002$).

Determination of IL-6 and NLR Cut-Offs as Predictors of COVID-19 Patient Mortality

A comparative study of the IL-6 and NLR findings was performed to obtain the ROC curve results, as shown in Figure 1.

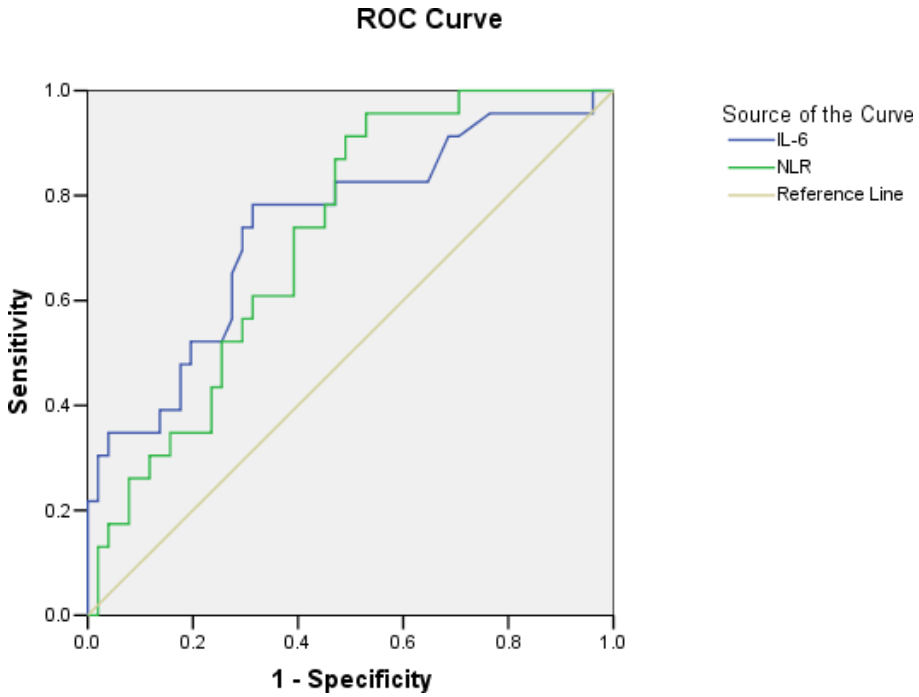

Figure 1. ROC curve for IL-6 and NLR examination results

The ROC curve determined the cut-off values for NLR and IL-6 with the highest sensitivity value and the lowest 1-specificity value. In addition, the sensitivity and specificity values, positive predictive value (NDP), and negative predictive value (NDN) were obtained in Table 4.

Table 4. IL-6 and NLR cut-off values as predictors of COVID-19 patient mortality

Variable	AUC	Cut-off value	Sensitivity	Specificity	NDP	NDN	p
IL-6 (pg/mL)	0.740	42.00	0.739	0.706	0.531	0.857	0.001#
NLR	0.721	5.51	0.739	0.608	0.459	0.838	0.002#

Note: #Significant at $p < 0.05$

The AUC value of IL-6 in COVID-19 patients was 0.740, meaning IL-6 levels could predict 74.0% of patient mortality. The cut-off value of IL-6 was 42.00 with a sensitivity of 0.739, meaning the likelihood that patients with an IL-6 level of >42.00 died was 73.9%. The specificity value of IL-6 was 0.706, meaning the likelihood of survival of patients with an IL-6 level of <42.00 was 70.6%. The NDP value was 0.531, meaning 53.1% of patients with an IL-6 level of >42.00 were likely to die. The NDN value was 0.857, meaning 85.7% of patients with an IL-6 level <42.00 were likely to survive. Thus, IL-6 can be used as a predictor of mortality ($p = 0.001$).

Examination of the NLR sample showed an AUC value of 0.721, indicating that the NLR value could predict 72.1% of patient mortality. The cut-off value of the NLR as a predictor of mortality was 5.51 with a sensitivity of 0.739, which indicated a likelihood of 73.9% that patients with an NLR of >5.51 would die. The specificity value of the NLR was 0.608, meaning the likelihood of a survival outcome in patients with an NLR value of <5.51 was 60.8%. The NDP was 0.459, meaning if the NLR was >5.51, there was a probability that 45.9% of those patients would die. The NDN was 0.838, meaning if the NLR was <5.51, there was a probability that 83.8% of those patients would survive. Thus, similar to IL-6, the NLR can be used as a predictor of mortality ($p = 0.002$).

The cross-tabulation results to determine the odds ratios for the cut-off values of NLR and IL-6 as mortality predictors in COVID-19 are shown in Table 5.

Table 5. Cross-tabulation analysis of NLR and IL-6 as predictors of mortality

Variable	Survivors (n = 51)	Non-survivors (n = 23)	OR (95% CI)	p
IL-6				
<42.00	36	6	Ref.	
≥42.00	15	17	6.80 (2.24–20.61)	<0.001#
NLR				
<5.51	31	6	Ref.	
≥5.51	20	17	4.39 (1.48–13.03)	0.006#

Note: #Significant at $p < 0.05$

The OR value of the IL-6 examination was 6.80 (95% CI 2.24–20.61; $p < 0.001$), meaning IL-6 was significantly related to COVID-19 patient outcome and patients with an IL-6 level of >42.00 had a risk of dying 6.80 times that of patients with an IL-6 level of <42.00. Therefore, the IL-6 level can be used as a mortality predictor,

The OR value of the NLR examination was 4.39 (95% CI 1.48–13.03; $p < 0.001$), meaning the NLR had a significant correlation with the COVID-19 outcome and patients with an NLR >5.51 had a risk of dying 4.39 times that of patients with an NLR <5.51. Thus, the NLR can be used as a mortality predictor.

Correlation of IL-6 with NLR in COVID-19 Patients

The correlation of IL-6 with NLR in COVID-19 patients was tested using the Spearman rank test because the data did not meet the normality assumption. The correlation between IL-6 and NLR in COVID-19 patients is shown in Table 6.

Table 6. Correlation of IL-6 with NLR

Variable	NLR	
	r (correlation coefficient)	p
IL-6	0.164	0.164

Note: #Significant at $p < 0.05$.

As shown in Table 6, the correlation between IL-6 and NLR in COVID-19 patients had an r-value of 0.164. The p-value was 0.164 ($p > 0.05$), indicating that this study's correlation between IL-6 and NLR in COVID-19 patients was not statistically significant. Although the relationship was positive and weak, there was a tendency that the higher the IL-6, the higher the NLR, but this was not statistically significant.

DISCUSSION

This study was conducted at Dr. Moewardi General Hospital, Surakarta. In total, 74 hospitalized COVID-19 patients were recruited. The mean age was 53.07 ± 16.20 years old, and most patients were males (41, 55.4%). These results support the findings of Jurado, *et al*. (2020) and Luís, *et al*. (2020), in which the average age of the participants was 63 years old, and male participants predominated (59.8% and 54.2%, respectively).[7,8] Older age was associated with higher IL-6 than age <65 years old ($p < 0.001$). The presence of comorbid disease appears to be the main cause significantly associated with IL-6 levels in people aged >65 years old.[8] Another cause may be immunosenescence, a decrease in the adaptive and innate immune systems associated with ageing.[9,10] This phenomenon involves dysfunction of B and T lymphocytes; reduced naive lymphocyte release, lymphocyte sparing, proliferation capacity and function of effector lymphocytes, and memory lymphocyte population; fibrotic changes in the structure of lymph nodes; and dysregulation of cytokine synthesis.[11]

The analysis conducted in this study related to patient age and mortality found no statistically significant difference in age between patients who survived and those who died ($p = 0.411$). This finding could be due to bias because the elderly patients in the study did not remember how old they were and did not have or carry valid residence documents, especially elderly patients living in rural areas. The same results were also obtained in the studies of Ziuzia-Januszewska, *et al*. (2022) and Cunningham, *et al*. (2020), who found no significant age difference in mortality.[12,13] However, young adult patients are still believed to survive better because they have fewer risk factors, such as comorbidities.

In this study, hypertension (26 patients, 35.1%) and diabetes (17 patients, 20.3%) were the most common comorbidities, but we did not find differences in comorbid diabetes ($p = 0.305$) and hypertension ($p = 0.313$) between survivors and non-survivors. These results were similar to prior study that showed no significant differences in type 2 diabetes mellitus and hypertension, although the frequency of non-survivors was greater than survivors.[12] Meanwhile, several other studies have shown that the presence of diabetes is strongly associated with the risk of death, the use of mechanical ventilation, and the need for ICU care. Even in young adults, diabetes plays a role as a risk factor for more severe clinical COVID- 19.[14,15]

Hypertension in COVID-19 plays an important role. It regulates the renin–angiotensin–aldosterone system (RAAS), inflammation, and immune responses.[16] In addition, patients with hypertension experience increased expression of angiotensin-converting enzyme (ACE)2, also known as a receptor

for SARS-CoV-2 infection.[17] Further, it is hypothesized that hyperglycemia can increase the chance of virus entry into cells because the expression of ACE2 and the virus both require glucose.[18]

There was a significant difference in the degree of severity between survivors and non-survivors ($p < 0.001$), wherein patients who died tended to have severe (12.5%) or critical (87.5%) degrees of severity. This finding was similar to the study conducted by Arslan, *et al.* (2021), which found that patients with severe disease characterized by treatment in the ICU and mechanical ventilation tended not to survive compared with patients with mild or moderate COVID-19 (with a lower p-value).[19] Of 91 patients transferred to the ICU in that study, approximately 66% died of acute renal failure, acute liver dysfunction, multiple acute organ dysfunction, or acute bleeding diathesis.

In this study, IL-6 levels showed a statistically significant difference between survivor and non-survivor groups ($p = 0.001$). IL-6 levels in survivors were lower than those in non-survivors. The results of this study obtained a cut-off value of 42.00 pg/mL for IL-6 as a mortality predictor, with a 73.9% sensitivity and 70.6% specificity. In addition, based on Table 5, it can be concluded that IL-6 can be used as a mortality predictor. Patients with an IL-6 level >42.00 pg/mL had a 6.80 times greater risk of dying than patients with an IL-6 level <42.00 pg/mL. This is similar to the study by Zhang, *et al*. (2020), in which an IL-6 concentration higher than 37.65 pg/mL predicted mortality with a sensitivity of 91.7% and a specificity of 95.7%.[20] As for patients treated in the ICU, Youden's index identified an IL-6 threshold of >406 pg/mL, which could be used as a mortality predictor (75% sensitivity and 64% specificity).[21]

COVID-19 induces the host immune response, which can clear the virus in most patients. The binding of the virion with viral nucleic acid receptors on lung epithelial cells and alveolar macrophage activation induces the release of cytokines and inflammatory mediators (IL-6, interleukin 1, and tumor necrosis factor-α). Then, neutrophils, monocytes, and many other immune cells are recruited. Cytokines also stimulate the bone marrow to release immature granulocytes and infiltrate the lungs, increasing the inflammatory response.[22,23] Elevated IL-6 levels in patients with COVID-19 are considered the most objective representation of a "cytokine storm." IL-6 binds to its receptor in the cell membrane (cis-signaling) or the soluble form (trans-signaling), and then activates the Janus kinase (JAK) pathway. This pathway leads to the maturation of effector T cells, increased vascular endothelial growth factor (VEGF) expression, increased vascular permeability, and decreased myocardial contractility, all contributing to organ damage and increased risk of death.[24]

The results of the statistical tests in this study found a difference in the NLR between survivors and non-survivors ($p = 0.002$, $p < 0.05$). NLR levels in survivors, with a median of 4.61 (0.13–50.26), were lower than those in non-survivors, with a median of 8.01 (2.77–28.52). This study obtained a cut-off value as a mortality predictor 5.51 (sensitivity of 73.9%; specificity of 60.8%). This study also showed that NLR had a significant relationship with the outcome of COVID-19 patients. Therefore, NLR could be used as a mortality predictor (OR 4.39, 95% CI 1.48–13.03; $p < 0.001$).

A study in Ethiopia found a higher cut-off value for NLR as a mortality predictor of 9.47 (sensitivity of 88.7%; specificity of 95.4%).[25] Another study found a cut-off value for NLR as a mortality predictor of 5.94, with 62% sensitivity and 64% specificity.[26] A meta-analysis involving 38 articles showed that patients with severe or critical COVID-19 who did not survive had NLR values at admission higher than those of non-severe COVID-19 patients who survived. Regardless of the NLR cut-off value, patients with elevated NLR levels had a greater risk of death, with a risk ratio of 2.74 (95% CI 0.98–7.66).[27]

A positive relationship between IL-6 and NLR was obtained in the correlation analysis. The higher the IL-6 level, the higher the NLR value. However, this relationship was weak, with a correlation coefficient of 0.164. Another study also found that IL-6 positively correlated with neutrophil count and NLR, but IL-6 negatively correlated with the lymphocyte count.[28] Meanwhile, Purwati, *et al.* (2021) found no correlation between IL-6 and NLR.[29]

Based on an autopsy study, IL-6 is directly involved in decreasing lymphocytes in COVID-19.[30] The acute-phase proteins induced by IL-6 can induce pro-inflammatory and anti-inflammatory mediators.[22,31,32] In addition, an increasingly severe inflammatory response may induce cytokine-induced apoptosis of lymphocytes.[33] Direct viral infection of ACE2-expressing lymphocytes, lymphatic organ destruction, and increased lymphocyte consumption in infected tissues are also possible causes of lymphocytopenia in COVID-19.[34,35]

LIMITATIONS

This study demonstrated the sensitivity and specificity of IL-6 and NLR as predictive biomarkers for COVID-19. However, there was a limitation in control bias because patients with diabetes mellitus or other infectious conditions that may affect IL-6 levels were not excluded. It is recommended to control this

tendency strictly in further studies. Moreover, bias can be minimized by increasing the number of study samples.

CONCLUSION

Aside from being as sensitive as NLR in predicting mortality in COVID-19 patients, IL-6 is more specific. This study found a positive correlation between IL-6 levels and NLR. The higher the NLR, the more likely IL-6 is also high, indicating a higher risk of mortality from COVID-19.

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None declared.

Conflict of Interest
The authors declared there is no conflict of interest.

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Authors' Contributions
Conceptualizing and initiating the study: HH. Collecting and analyzing data (interpretation): HH, JA, AA, and ASL. Drafting and making manuscript: HH, AG, and HS. Critical revision of the manuscript: HH and HS. All authors reviewed and approved the final version of the manuscript.

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