The Effect of Neutrophil Lymphocyte Ratio and Bacterial Coinfection on Mortality and Length of Hospital Stay of COVID-19 Patients

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Article Info	Abstract
	Background: Systemic inflammation caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results in high mortality. The presence of bacterial coinfection in COVID-19 patients contributes to the clinical severity. The Neutrophil-Lymphocyte Ratio (NLR) is a biomarker that provides essential information about systemic inflammation status in COVID-19. **Objective:** This study aims to retrospectively analyze the effect of NLR and bacterial coinfection on mortality and length of hospital stay of COVID-19 patients based on the clinical data of COVID-19 patients treated at Dr. Moewardi General Hospital in Surakarta. **Methods:** This cross-sectional study analyzed secondary data from 87 adult COVID-19 patients' Medical Records at Dr. Moewardi Hospital in August 2021. The eligibility criteria included adult patients aged 18 years or above who had been diagnosed with COVID-19 and hospitalized. Patients with pregnancy, HIV/AIDS, malignancy, stage 5 chronic kidney disease, as well as patients with incomplete medical records were excluded. **Results:** The study strongly correlated bacterial coinfection and NLR (r= 0.528). It was also found that NLR and bacterial coinfection simultaneously affected the length of hospital stay (Sig.= 0.02) and mortality (Sig.= 0.027). **Conclusion:** NLR and bacterial coinfection affect mortality and length of hospital stay of COVID-19 patients. Higher NLR and bacterial coinfection contribute to higher mortality and more extended hospitalization.

INTRODUCTION

Since December 2019, the world has faced a global health crisis triggered by the COVID-19 pandemic caused by a new type of coronavirus, SARS-CoV-2. Indonesia was one of the countries that were severely affected by the COVID-19 pandemic. The first COVID-19 case in Indonesia was found on 02 March 2020. From July to August 2021, the COVID-19 death rate in Indonesia rose to 350%. As of 19 January 2022, the number of confirmed COVID-19 cases in Indonesia was 4,273,783, with 9,564 active cases and 144,183 deaths[1].

COVID-19 has a broad spectrum of clinical manifestations. The clinical manifestation of COVID-19 can be grouped based on their clinical symptoms into asymptomatic, mild, moderate, or severe[2]. Moderate and severe COVID-19 have been linked with high mortality rates and lengthy hospitalizations. Several studies have shown that old age, comorbidities (hypertension and cardiovascular disease), SOFA score, and several laboratory indices, such as interleukin (IL-6) and C-reactive protein (CRP), were associated with a poorer prognosis[3]. However, some laboratory tests required for determining COVID-19 risk factors were complex and expensive. Therefore, an indicator or clinical marker that is cheap, simple, easy to find, has a fast turnaround time, and is sensitive and specific is needed. One of the markers that met the abovementioned criteria is the Neutrophil Lymphocyte Ratio (NLR) from routine blood test[4].

NLR is a biomarker that provides essential information about systemic inflammation[5]. Increased NLR is associated with mortality in patients with infectious diseases[6]. NLR is calculated from routine blood tests by dividing the absolute neutrophil count by the absolute lymphocyte count[7]. A systematic review and meta-analysis by Feng *et al.* addressing inflammatory parameters in COVID-19 infection mention that moderate to severe COVID-19 patients had higher NLR values than mild COVID-19 patients. In addition, there was also a higher NLR value in severe COVID-19 patients who died compared to those who recovered. It was necessary to investigate whether NLR is associated with mortality and length of stay in COVID-19 patients[8].

Bacterial coinfection is commonly identified in viral respiratory tract infections[9]. The rate of bacterial coinfection in SARS and MERS patients was 11%, considered a significant cause of morbidity and mortality[10]. Considering these data, several researchers have shown that COVID-19 patients were more susceptible to bacterial coinfection. However, the association between bacterial coinfection in COVID-19 patients and the disease prognosis is still not well-reached[11].

Research related to COVID-19 has focused more on the epidemiology and clinical features of the patients rather than on the risk factors and prognosis. Therefore, this study aims to retrospectively analyze clinical data of COVID-19 patients treated at Dr. Moewardi General Hospital to determine the relationship between NLR and bacterial coinfection with mortality and length of stay of COVID-19 patients. The findings of this study are expected to improve diagnostic approaches to identify high-risk patients and to determine appropriate interventions to reduce mortality and shorten the length of hospital stay.

METHODS

This research was an analytical observational study with a cross-sectional design using secondary data from 87 adult COVID-19 patients at Dr. Moewardi General Hospital in August 2021. The inclusion criteria in this study were patients diagnosed with COVID-19 over 18 years old who were hospitalized at Dr. Moewardi General Hospital in August 2021. The exclusion criteria in this study were pregnant women, patients with comorbidities (HIV/AIDS, malignancy, stage 5 chronic kidney disease undergoing hemodialysis), as well as patients with incomplete medical record data.

The sampling method used was purposive sampling. The independent variables in this study were the Neutrophil Lymphocyte Ratio (NLR) at arrival and the incidence of bacterial coinfection. The dependent variables in this study were mortality and length of hospital stay of COVID-19 patients. The confounding variables in this study were comorbid diseases, namely hypertension and type 2 diabetes mellitus. The data was processed manually using the Statistical Product and Service Solution (SPSS) program version 26.0. The correlation tests used in this study were point biserial, multiple linear, and multiple logistic regression.

This research is ethically approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital with ethical clearance number 465/IV/HREC/2022.

RESULTS

This study involved 87 eligible patients. Three patients from 90 did not meet the inclusion criteria due to incomplete medical record data.

Data was collected from May to July 2022 using medical record data for COVID-19 patients at Dr. Moewardi General Hospital in August 2021. From 87 patients, the average NLR was 8.41, and the average length of hospital stay was eight days. The distribution of research data characteristics is shown in Table 1, and the distribution of comorbidities is described in the figure1.

The point biserial test determined the relationship between NLR and bacterial coinfection. The coefficient gained was interpreted based on the De Vaus correlation coefficient interpretation. The result indicated that the NLR value in this study has a strong relationship with bacterial coinfection (p=0.528). The multiple linear regression test to determine the effect of NLR and bacterial coinfection on length of hospital stay showed a significant result (p=0.020). Further T-test analysis was performed to see the impact of the NLR and bacterial coinfection on the length of hospital stay. The T-test showed a significant effect of NLR on the length of hospital stay (p=0.045).

On the other hand, the T-test showed that bacterial coinfection did not significantly affect the length of hospital stay (p= 0.529). Moreover, multiple logistic regression tests to determine the effect of NLR and bacterial coinfection on mortality also indicated a significant result (p=0.027). A chi-square test was conducted to examine whether NLR and bacterial coinfection influence the mortality of COVID-19 patients. The result showed a critical value of 0.168 for NLR and bacterial coinfection.

Table 1. Characteristics of Research Sample Data

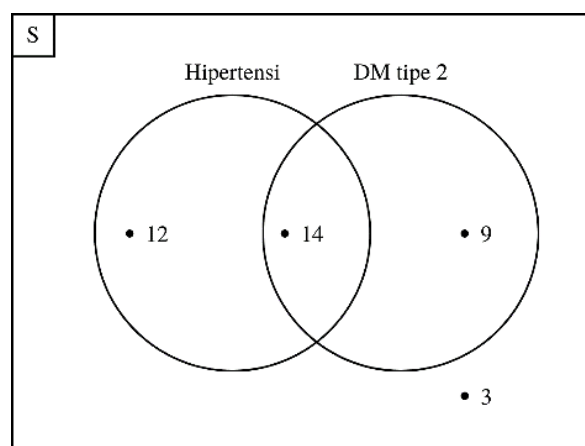| No	Characteristics	Frequency	Percentage (%)
1	NLR		
	≤3,3	24	27,6
	≥3,3	63	72,4
2	Bacterial coinfection		
	No	50	57,5
	Yes	37	42,5
3	Length of hospital stay		
	≤8 days	55	63,2
	>8 days	32	36,8
4	Mortality		
	Alive	65	74,7
	Dead	22	25,3
5	Disease severity degree		
	Mild	0	0
	Moderate	53	60,9
	Severe	34	39,1
6	Comorbidity		
	No	49	56,3
	Yes	38	43,7

Figure **1.** Venn Diagram Comorbidity Analysis

DISCUSSION

In the univariate analysis of the variables, NLR was categorized as ≤ 3.3 and > 3.3 based on the results of research conducted by Pramana *et al.,* which stated that COVID-19 patients with NLR value below 3.3 indicate better prognosis and an NLR value above 3.3 indicates poorer prognosis[12]. In this study, there were 24 patients (27.6%) with NLR ≤ 3.3 and 63 patients (72.4%) with NLR > 3.3. Eslamijouybari

et al. stated that NLR values were higher in COVID-19 patients with moderate to severe symptoms compared to COVID-19 patients with mild symptoms[13]. The high percentage of COVID-19 patients with NLR > 3.3 in this study followed the data on the disease severity in this study. This study recorded that all patients showed moderate (60,9%) and severe clinical symptoms (39.1%). The average NLR value among all patients involved in this study was 8.41. This result agrees with the research results conducted by Toori *et al.*, where the average NLR value in moderate to severe COVID-19 patients was 4.79-9.97[14]. The NLR value indicated an immune response toward infection. The higher Neutrophile count over the lymphocyte suggests the severity of inflammation. Neutrophiles are responsible for early adaptive response toward infections, including viral infection. Neutrophils secrete pro-inflammatory and immunomodulatory cytokines in response to early infection[15]. The release of pro-inflammatory cytokines might contribute to systemic inflammation, which underlies the clinical severity of COVID-19 in patients.

This study reported bacterial coinfection in 37 patients (42.5%). The COVID-19 patients were considered to have bacterial coinfection if they had positive results on bacterial cultures from biological samples (urine, blood, and pus) or had a leukocyte value above 10,000 cells per microliter. From 87 patients, culture examination was only performed on 14 patients (16%). The biological sample for culture examination was taken on the first day of patient admission. The culture examination of 16 patients gave ten positive results, namely three positive results from urine culture, six positive blood culture results, and one positive pus culture result. Compared with previous research conducted by Langford *et al.*, which shows that the percentage of bacterial coinfection in SARS and MERS patients was 11%, this study indicates that COVID-19 has a higher rate of bacterial coinfection of 42.5%[10]. This phenomenon could happen because Dr. Moewardi General Hospital is a national COVID-19 referral center. Thus, most of the patients who were referred to the hospital had comorbidities, including bacterial coinfection.

The average hospital stay of COVID-19 patients involved in this study was eight days. This result is not much different from the average length of stay for COVID-19 patients based on a survey by Vekaria *et al.*, which was 8.4 days[16]. The policy for hospital discharge of COVID-19 patients treated in Dr. Moewardi General Hospital might explain the average length of hospital stay. The high referral cases of COVID-19 patients to Dr. Moewardi General Hospital in August 2021 underlies the hospital policy to send the patients for home care. They are considered to survive the critical phase, are clinically stable, and are not receiving antiviral anymore.

The mortality rate of the patient in this study population was 25.3%. These results were not much different from the mortality percentage of hospitalized COVID-19 patients in the study conducted by Perazzo *et al.*, which was 22%[17]. The clinical severity of patients at hospital admission might be responsible for the mortality rate. Similar to the study reported by Perazzo *et al.* in Brazil, the COVID-19 patients referred to Dr. Moewardi Hospital were recognized in moderate and severe conditions.

This study's univariate analysis of comorbidities showed 49 patients (56.3%) without comorbidities and 38 patients (43.7%) with

comorbidities. There were 26 patients with hypertension, 23 patients with type 2 diabetes mellitus, and three patients with other comorbidities, namely hepatitis B and C, stroke, and anemia. The severity and mortality of COVID-19 were both significantly heightened with the presence of type 2 diabetes mellitus and hypertension[18]. The average NLR of the population without comorbidities was 7.3, and that of those with comorbidities was 9.4. Following bacterial coinfection, the higher NLR ratio among patients with comorbidities might correlate with increased neutrophils as part of the innate immune response toward bacterial coinfection. Bacterial infection was considered the cause of high neutrophile count. Furthermore, bacterial coinfection might also induce systemic inflammatory stress in COVID-19 patients, which results in a decrease in lymphocyte count due to the increase in lymphocyte apoptosis[19].

The bivariate analysis between bacterial coinfection and NLR with the point biserial test found a strong positive correlation between both variables, as proven by the coefficient value between $0.50 - 0.69$ (r = 0.528). These results follow the research conducted by Vélez-Páez *et al.*, which explains the correlation of increased NLR in COVID-19 patients with bacterial coinfection. During bacterial coinfection, Pathogen-Associated Molecular Patterns (PAMPs) such as lipopolysaccharides (LPS) bind to Pattern Recognizing Receptors (PRRs) and activate immune responses by the production of pro-inflammatory interleukin (TNF alpha, IL6, IL-1, and IL-8) and accelerates T cell apoptosis, which induces neutrophil activation and output as well as a decrease in lymphocytes in the blood[20].

The multivariate analysis to determine the effect of NLR and bacterial coinfection on the length of stay of COVID-19 patients with multiple linear regression tests gained a significant level of 0.02 (Sig. < 0.05), which means that NLR and bacterial coinfection simultaneously have an influence on the length of hospital stay in COVID-19 patients. A t-test was performed to see the effect of the two independent variables separately. The T-test showed a significant level of NLR of 0.045 (Sig. < 0.05) and a substantial level of bacterial coinfection of 0.529 (Sig. > 0.05). It can be concluded that, independently, NLR affects the length of hospital stay, while bacterial coinfection does not affect the length of stay of COVID-19 patients. These results follow the theory proposed by Yang *et al.*, which states that an increase in the NLR value could exacerbate disease symptoms and prolong the duration of hospitalization for COVID-19 patients[21].

The multiple logistic regression test was conducted to determine the effect of NLR and bacterial coinfection on the mortality of COVID-19 patients. A significant level of 0.027 (Sig. < 0.05) was obtained. It can be concluded that NLR and bacterial coinfection simultaneously affect mortality. However, when testing the influence of the two independent variables separately using the Chi-square test, the results obtained a significant level of NLR of 0.168 (Sig. > 0.05) and a substantial level of bacterial coinfection of 0.168 (Sig. > 0.05), which means that both bacterial coinfection and NLR independently do not affect mortality. This result contradicts the theory proposed by Silva *et al.* and a systematic review and meta-analysis by Feng *et al.* Silva *et al.* explained that bacterial coinfection in SARS-CoV-2 infection

led to a worse prognosis due to increased oxidative stress, which could decrease the host immune response and increase the inflammatory response, which could cause tissue damage, worsen the course of the disease, and increase mortality[22]. Feng *et al*. said that COVID-19 patients with severe clinical symptoms who died had higher NLR values than those who recovered[8]. The different results in this study were influenced by the limited number of samples and the limited performance of bacterial culture.

CONCLUSION

NLR and bacterial coinfection affect mortality and length of stay of COVID-19 patients at Dr. Moewardi General Hospital. The result of this study indicated the potential use of the NLR as a predicting marker for COVID-19 clinical outcomes. The findings of this study are expected to improve diagnostic approaches to identify high-risk patients and to determine appropriate interventions to reduce mortality and shorten the length of hospital stay.

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