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Neutrophil-to-Lymphocyte Ratio and Comorbidities as Mortality Predictors for COVID-19 Patient at Dr. Moewardi Hospital Surakarta

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ABSTRACT

The COVID-19 pandemic has drawn global attention as its main health issue. The rapid transmission and the diverse degree of severity have caused complicacy in controlling the disease. A hematological lab test has been a standard procedure done during therapy. This study aimed to determine the relation of the hematological parameter as a COVID-19 mortality predictor. The cohort retrospective method was used for this study by observing the medical records of critically ill COVID-19 patients admitted at Dr. Moewardi Hospital, Surakarta, from May 2020 to June 2021. The observed variables in this study were age, gender, comorbidities, and hematological lab test towards the outcome. The results were then analyzed bivariate and multivariate with SPSS. Out of 161 data, 101 were found alive and 60 deceased. Bivariate analysis showed that age of 50-80 years (RR= 2.246; p=0.029), comorbidities (RR=2.891; p=0.008), leucocyte>9850/µl (RR=2.634; p=0.004), neutrophil percentage >84.25% (RR=4.808; p=0.000), lymphocyte percentage<22% (RR=0.065; p=0.008), and NLR>9.326 (RR=5.031; p=0.000) had a relationship with the outcome. Gender, hemoglobin level, and platelet did not significantly correlate with the patient's outcome. Multivariate analysis showed that a history of comorbidities (RR=2.9326; p=0.012) and NLR >9.326 (RR=5.073; p=0.000) were proven to be a good predictor for mortality of COVID-19 patients. This result can be advantageous for clinicians in predicting the mortality of COVID-19 patients.

Keywords: Hematological parameter, mortality predictor, COVID-19

INTRODUCTION

Lately, SARS-CoV-2 has caused a global shock worldwide through a pandemic. On April 27, 2020, the case fatality rate of COVID-19 in Indonesia reached 8.7%.[1] The typical symptoms of COVID-19 are fever, cough, fatigue, and anorexia, which are later classified into categories such as mild, moderate, severe, and critical.[2] In critically ill patients, Acute Respiratory Distress Syndrome (ARDS), sepsis, and septic shock are frequently found.[2] The leading causes of death of COVID-19 patients are the deteriorating condition of the patient and cytokine storm.[3] Organ failure such as respiratory distress (94%), shock (81%), and ARDS (74%) can also occur and cause mortality.[4]

SARS-CoV-2 infects the host via ACE2 receptors, transforming angiotensin 2 to other conformation. The decline of ACE2 receptors will cause an increase in angiotensin 2, leading to vasoconstriction, inflammation, and fibrosis to the extent of lung damage.[5] SARS-CoV-2 causes immune dysregulation and the production of inflammatory cytokines such as TNF-α, IL-6, IL-2R, and IL-10. Excessive production of cytokines will lead to cytokine storms.[6]

Previous studies regarding factors affecting COVID-19 prognosis are smoking, age >60 years, history of non-communicable diseases such as hypertension, diabetes, heart disease, cerebrovascular disease, and immunosuppressive state.[2] The case fatality rate of COVID-19 varies in each country and can be altered by the patient's age, history of comorbidities and the severity of COVID-19.[7] Changes in laboratory test results such as the presence of leukocytosis, lymphopenia, thrombocytopenia, and increased NLT are also capable of affecting the prognosis of COVID-19.[8] Therefore, this research was performed to determine the use of routine hematological to predict mortality of COVID-19 patients.

METHODS

This study was analytical observational research with a cohort retrospective approach. The study was conducted at Dr. Moewardi Hospital, Surakarta, from June to August 2021. The samples were secondary

data and were taken with a total purposive sampling method. The data were medical records of COVID-19 patients admitted from May 2020 to June 2021, which met the inclusion and exclusion criteria. The inclusion criteria were patients aged 18-80, diagnosed with COVID-19, and categorized as critically ill (determined by PaO2/FiO2<300 mmHg). The exclusion criteria were patients with a history of the hematological disease, a history of corticosteroid treatment, diagnosed with autoimmune diseases such as systemic lupus erythematosus, patients with a history of cancer, and patients with HIV, hepatitis B, and hepatitis C, patients with trauma and pregnant females. After the inclusion and exclusion process, the total number of subjects was 161.

The variables that were observed in this study were patient's outcome as a dependant variable; age, gender, and presence of comorbidities as confounder variables; and hematology parameters such as hemoglobin level, platelet count, leukocyte count, neutrophil percentage, lymphocyte percentage, and Neutrophil-to-Lymphocyte Ratio (NLR) as independent variables. Hematology lab test results were collected and standardized according to the International Council of Standardization in Haematology (ICSH) guideline. The clinical outcome was categorized into 2, dead or alive; age was categorized into patients 18-50 years old and 50-80 years old; and gender was categorized into 2, males or females. The presence of comorbidities was also categorized into 2, yes or no. The comorbidities are hypertension, diabetes, history of heart disease, stroke, previous lung disease, and chronic kidney disease. The hematological parameter was obtained as numeric data but later was categorized based on the cut-off value.

This study's analysis began with descriptive analysis of the categorical data to determine the distribution of the data and proceeded with correlation analysis using the Chi-Square test. Median, minimum, maximum, and Kolmogorov-Smirnov normality tests were also provided for the hematological parameter data. The cut-off value was then obtained using the Receiver Operating Characteristics (ROC) curve for the numeric data. The most suitable cut-off value was determined by analyzing the sensitivity and specificity. After grouping, the categorical variables were later analyzed bivariate using a simple logistic regression test. Variables that showed significant correlation with the dependent variable would then proceed with a multivariate analysis using a multivariate logistic regression test in a stepwise backward method. All analysis was performed using IBM SPSS Statistics ver 23.0.

This study has been declared ethically feasible by the Research Ethics Committee of Dr. Moewardi Hospital, Surakarta, with number 390/IV/HREC/2021.

RESULTS AND DISCUSSIONS

There were 161 patients observed for this study, which consisted of 101 alive and 60 deceased subjects. Tables 1 and 2 show the data distribution of the subjects.

A ROC curve for hematological parameters was performed to determine the cut-off value (Figure 1). The curve shows that leukocyte, neutrophil percentage, and NLR could be good predictors for COVID-19 mortality (Table 3). On the contrary, hemoglobin level, platelet count, and Area Under the Curve (AUC) of lymphocyte show insignificance as predictors. For the three insignificant variables, the suggested cut-off value from the clinical pathology laboratory of Dr. Moewardi Hospital was then used instead of ROC's cut-off value.

Table 1. The characteristics of subjects

Clinical Characteristics	Critically Ill COVID-19 Patient		p-value*
	Alive n(%)	Deceased n(%)	
Age			0.026
18–50 years old	33 (32.7)	10 (16.7)	
50¯80 years old	68 (67.3)	50 (83.3)	
Gender			0.764
Males	63 (62.4)	36 (60)	
Females	38 (37.6)	24 (40)	
Comorbid			0.007
Absent	37 (36.6)	10 (16.7)	
Presence	64 (63.4)	50 (83.3)	

*p-value <0.05 was significant (Chi-Square test)

Table 2. Characteristics of hematological parameters

Hematological Parameters	Critically Ill COVID-19 Patient		
	Median	Min-Max	p-value*
Hemoglobin (g/L)	129	46-189	0.019
Leukocyte (10^9/L)	96	3-153.3	0.000
Platelet (10^9/L)	235	19-808	0.006
Neutrophil (%)	83.1	25-97.5	0.000
Lymphocyte (%)	9.6	0.46-64	0.000
NLR	8.594	0.43-188.5	0.000

*p-value >0.05 was significant (Kolmogorov-Smirnov normality test)

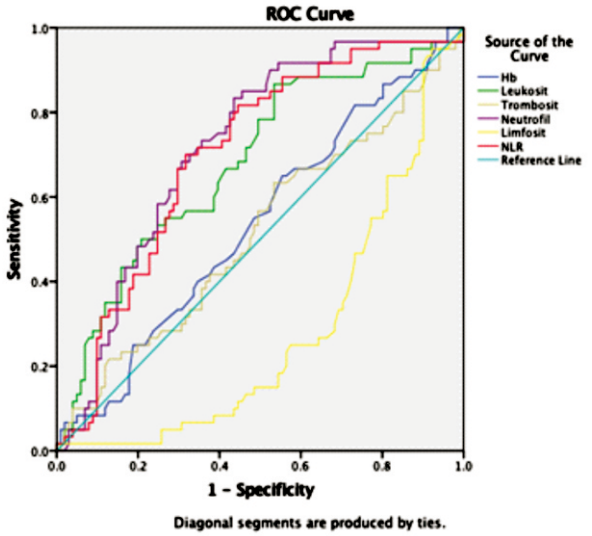

Figure 1. ROC curve of hematological parameters

After ROC, the cut-off value of NLR showed a high value. The higher value of NLR was obtained because all the observed subjects were critically ill, compared to an average person reference. The cut-off values for hemoglobin, platelet, and lymphocyte were 120 g/dL, 150x109/L, and 22%, respectively. The variables were then categorized into two groups (Table 4).

All confounder and independent variables were tested with a simple logistic regression test (Table 5). Out of all nine variables, six variables (age 50-80 years old, comorbidities, leukocyte count >9.85x109/L, neutrophil percentage >84.25%, lymphocyte percentage >22%, and NLR >9.326) were found to be significant and can be used to predict mortality.

Table 4. Data distribution of hematological parameter

Hematological Parameter	Critically III COVID-19 Patient		p-value*
	Alive n (%)	Deceased n (%)	
Hemoglobin (g/L)			0.698
≤120	35 (34.7)	19 (31.7)	
> 120	66 (65.3)	41 (68.3)	
Leukocyte (10^9/L)			0.004
≤9.85	61 (60.4)	22 (36.7)	
>9.85	40 (39.6)	38 (63.3)	
Platelet (10^9/L)			0.613
≤150	17 (16.8)	12 (20.0)	
>150	84 (83.2)	48 (80.0)	
Neutrophil (%)			0.000
≤84.25	68 (67.3)	18 (30.0)	
>84.25	33 (32.7)	42 (70.0)	
Lymphocyte (%)			0.001
≤22	80 (79.2)	59 (98.3)	
>22	21 (20.8)	1 (1.7)	
NLR			0.000
≤9.326	69 (68.3)	18 (30.0)	
>9.326	32 (31.7)	42 (70.0)	

*p-value significant if <0.05 (Chi-Square test)

This study showed that patients in the 50-80 age group had a higher risk of mortality (2.246; p=0.029). This result was consistent with a meta-analysis study by Biswas *et al.*[9] The declined immune system in the elderly, and an increase in ACE2 receptor expression caused the increased mortality.[10] The presence of comorbidities among the subjects also increased mortality by 2.891 (p=0.008). A condition in which comorbid is present will cause decreases in immune function.[9]

Regarding patient mortality, leukocyte count and neutrophil percentage changes can be figured out.

Table 3. The cut-off value of the hematological parameter

Variables	Area Under the Curve (CI 95%)	p-value	Cut-off Value	Sensitivity	Specificity
Hemoglobin (g/L)*	53.5% (44.4%-62.7%)	0.453	128.5	55.0%	51.5%
Leukocyte (10^9/L)	68.1% (59.5%-76.7%)	0.000	9.85	63.3%	60.4%
Platelet (10^9/L)*	51.6% (42.2%-61%)	0.740	234.5	53.3%	51.5%
Neutrophil(%)	72.1% (64.1%-80.1%)	0.000	84.25	70.0%	67.3%
Lymphocyte(%)*	27.9% (20%-35.7%)	0.000	8.6	33.3%	30.7%
NLR	70.2% (62%-78.4%)	0.000	9.326	70.0%	68.3%

*Cut-off values from the Clinical Pathology laboratory of Dr. Moewardi Hospital were used for the variable due to insignificant results (AUC<50% or p>0.05)

Table 5. Bivariate analysis using a simple logistic regression test between independent and confounding variables toward a clinical outcome

Variables	RR (95% CI)	p-value
Age		
18-50 years old	1	
50-80 years old	2.246 (1.094-5.380)	0.029*
Gender		
Males	1	
Females	1.105 (0.574-2.127)	0.765
Comorbid		
Absent	1	
Presence	2.891 (1.311-6.372)	0.008*
Hemoglobin		
≤ 120 g/L	0.874 (0.442-1.727)	0.698
> 120 g/L	1	
Leukocyte		
≤ 9.85 (10^9/L)	1	
> 9.85 (10^9/L)	2.634 (1.362-5.093)	0.004*
Platelet		
≤ 150 (10^9/L)	1.235 (0.544-2.804)	0.613
> 150 (10^9/L)	1	
Neutrophil		
≤ 84.25%	1	
> 84.25%	4.808 (2.409-9.598)	0.000*
Lymphocyte	0.065 (0.008-0.494)	0.008*
≤ 22%	1	
> 22%		
NLR		
≤ 9.326	1	
> 9.326	5.031 (2.515-10.064)	0.000*

*p-value <0.05 was significant, variables were then analyzed using a multivariate logistic regression test

This study proved this, which showed that leukocytes>9.85x109/L had a risk ratio of 2.634 (p=0.004). Increased white blood cell count is the consequence of increased neutrophil.[11] The neutrophil percentage >84.25% was also proven to contribute to mortality by 4.808 (p=0.000). Contrary to other studies, neutrophils were the only white blood cell component that increased in COVID-19 patients.[11] The other white blood cells component will decrease due to the SARS-CoV-2 reaction to the ACE2 receptor in white blood cells.[12] Neutrophil plays a role as a first-line guard for the host in fighting SARS-CoV-2. Neutrophil has a unique capability called Neutrophil Extracellular Trap (NET), which will give an anti-inflammatory effect by degrading cytokines and chemokines. However, it can also have a proinflammatory effect due to extensive tissue damage caused by the extravasation of viruses from the damaged cells.[13] Lymphocyte percentage <22% indicated 0.065 times higher protection (p=0.008). However, other studies showed that lymphopenia increased mortality.[14] Lymphopenia in the COVID-19 patient occurs due to increased apoptosis caused by FasL, TNF-α, and TRAIL.[15]

Gender did not significantly affect mortality, in contrast to a study in China, which found that 2.4 times increased mortality was reported in males compared to females.[16] However, the results may change due to demographic factors and data distribution.[16] Hemoglobin <120 g/L and >120 g/L did not show a significant difference in mortality risk despite a finding by other studies suggesting that anemia can increase mortality due to the host's declining ability to transfer oxygen within the circulation.[17] Another insignificant variable was platelet count <150x109/L. Thrombocytopenia in COVID-19 might happen due to side effects from treatment with Hydroxychloroquine, Azithromycin, and Enoxaparin.[18] On the contrary, another study suggested that respiratory distress might cause endothelial damage and activate the coagulation cascade, which produces a thrombus. A low platelet count might indicate increased coagulation activity that can increase mortality.[12]

Table 6. The multivariate logistic regression result

Variable	Adjusted RR (95% CI)	p-value
Age	1.411 (0.56-3.551)	0.465
Comorbid	2.936 (1.265-6.815)	0.012*
Leukocyte	1.061 (0.473-2.377)	0.886
Neutrophil	2.016 (0.656-6.191)	0.221
Lymphocyte	5.924 (0.727-48.258)	0.096
NLR	5.073 (2.494-10.320)	0.000*

*p <0.05 was significant

According to the multivariate logistic regression test, the variables that have significant results towards mortality are the presence of comorbidities NLR (Table 6). When tested simultaneously, the variables that can be a predictor for mortality altogether have a p-value>9.326. According to the ROC test result, the model for those two variables showed a significant impact of AUC=0.734 and can be concluded as a good predictor. A previous study also suggested that NLR was a risk factor for COVID-19.[19] Comorbidities will cause a downfall in the immune system.[9] In addition, increased NLR indicates neutrophilia and a decrease in lymphocytes. Increased NLR reveals immune dysregulation due to inflammatory response from

host to SARS-CoV-2.[20] Multivariate logistic regression in this study obtained a formula y= -2.147 + 1.077 (if comorbid was found) + (1.624xNLR) with y as a risk ratio for mortality in critically ill COVID-19 patients.

Categorizing comorbid variables into two groups instead of detailed observation of each variable remained one of the limitations of this study. In addition, the additional effect of multimorbidities and other comorbidities, such as obesity, was not observed in this study. Multicenter research was highly suggested for further study to improve the validity of this study. Another limitation was that the lab results from the COVID-19 patients were obtained one day before up to 3 days after admission to the intensive care unit. This limitation might be due to the massive number of patients compared to the limited capability of laboratory testing.

CONCLUSIONS AND SUGGESTIONS

Hematological parameters of leukocyte (>9.85x109/L), neutrophil percentage (>84.25%), lymphocyte percentage (<22%), and increased NLR (>9.326) were mortality predictors for critically ill COVID-19 patients. On the other hand, the hematological parameter of hemoglobin (<120 g/L) and platelet count (<150x109/L) were mortality predictors. It was suggested to perform further studies to analyze the types of mortality. Multicentered research was also proposed for further investigation to increase the validity of this study for a more comprehensive benefit in the treatment of COVID-19.

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