

Analysis of Neutrophil Lymphocyte Ratio and Absolute Lymphocyte Count as Predictors of Severity of COVID-19 Patients

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ABSTRACT

It is important to predict the severity of COVID-19 during the pandemic. Both Neutrophil Lymphocyte Ratio (NLR) and Absolute Lymphocyte Count (ALC) are two easy, low-cost, and fast inflammatory markers, which positively correlate with the severity of COVID-19. The purpose of this research was to analyze the value of NLR and ALC as predictors of COVID-19 severity. This research was a retrospective study using medical record data of 376 COVID-19 patients during April-September 2020 at the Hasanuddin University Hospital and Makassar City Regional Hospital. Patients were classified into non-severe and severe COVID-19. Neutrophil lymphocyte ratio and ALC values were determined based on routine blood test (Sysmex XS-800i) results, statistical analysis using Independent T-test, while NLR and ALC diagnostic values were analyzed with Receiver Operating Characteristics (ROC) curve to obtain the cut-off value, $p < 0.05$ was significant. The samples consisted of 372 non-severe and 49 severe COVID-19 patients. Neutrophil lymphocyte ratio value in non-severe (4.02 ± 5.22) was significantly different from severe COVID-19 (9.81 ± 7.06) ($p < 0.001$), similar to ALC in non-severe ($2.00 \pm 0.83 \times 10^3/\mu\text{L}$) and severe COVID-19 ($1.22 \pm 0.78 \times 10^3/\mu\text{L}$) ($p < 0.001$). Receiver operating characteristics curve showed that NLR had a sensitivity of 91.8% and specificity of 66.4% with a cut-off ≥ 3.17 with Negative Predict Value (NPV) of 98.2% and Positive Predict Value (PPV) of 29.0%; while ALC had a sensitivity of 81.6% and specificity of 64.8% at cut-off $\leq 1.74 \times 10^3/\mu\text{L}$ with NPV of 95.9% and PPV of 25.8%. Increased NLR and decreased ALC in severe COVID-19 patients occurred due to an increased inflammatory response resulting in a decreased cellular immunity. Receiver operating characteristics curve showed a cut-off for NLR of 3.17 and ALC of $1.74 \times 10^3/\mu\text{L}$, indicating an optimum sensitivity and specificity. It was concluded that NLR and ALC can be used as predictors of COVID-19 severity with a cut-off ≥ 3.17 and $\leq 1.74 \times 10^3/\mu\text{L}$, respectively.

Keywords: COVID-19, neutrophil lymphocyte ratio, absolute lymphocyte count, predictor

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an atypical pneumonia disease caused by infection with the coronaviridae class virus, which is a single positive RNA virus, and is known as Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2). COVID-19 was first discovered in December 2019 in Wuhan, China and then spread throughout the world, resulting in the announcement by WHO on March 11, 2020 declaring that COVID-19 is a pandemic in the world.¹⁻³

Based on scientific evidence, COVID-19 can be transmitted from human to human through airway droplets such as coughing and sneezing and fecal contamination and those who are most at risk of contracting this disease are people whose close contact with people infected with COVID-19. COVID-19 infection can cause mild to severe

symptoms. The main clinical symptoms that emerge are fever (temperature $> 38^\circ\text{C}$), cough and shortness of breath and may be accompanied by fatigue, myalgia and gastrointestinal symptoms such as diarrhea. In severe cases of COVID-19, worsening occurs rapidly and progressively such as Acute Respiratory Distress Syndrome (ARDS), septic shock, and metabolic acidosis, which is difficult to correct and dysfunction of bleeding or coagulation. In some patients, the symptoms appear mild, although in some cases no symptoms are observed and most patients have a good prognostic condition with a small proportion of them are classified into a critical condition and die.⁴⁻⁶

The number of COVID-19 cases increases quite rapidly, and it has spread to various countries in a short time. As of October 17, 2020 it was reported that the total number of confirmed cases of COVID-19 was 39,633,171 cases with a total

1,109,834 deaths (CFR 2.8%) in 215 affected countries. A total 357,762 confirmed cases were reported in Indonesia with 12,431 deaths (CFR 3.48%), while 17,346 confirmed cases with 442 deaths were reported in South Sulawesi.⁷

The diagnosis of COVID-19 is confirmed based on anamnesis to identify the history of traveling to endemic areas or a history of close contact with people infected with COVID-19, clinical symptoms that lead to pneumonia, radiological and laboratory tests. The gold standard to diagnose COVID-19 is the molecular identification of SARS-CoV-2 by using the Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-qPCR) or viral genome sequencing method. However, limited facilities and human resources cause the inability to carry out broad molecular identification in laboratories, leading to delayed diagnosis and management of COVID-19. One of the simple, fast, and widely available parameters are the hematological parameters that can determine the number and ratio of inflammatory cells.^{4,8-10}

Neutrophil Lymphocyte Ratio (NLR) has been reported to have a vital role in determination of inflammatory state in patients. Neutrophil lymphocyte ratio is the number of neutrophils divided by the number of lymphocytes. Under physiological stress, the number of neutrophils increases, while the number of lymphocytes decreases. Neutrophil lymphocyte ratio combines these two changes, making it more sensitive than neutrophils or lymphocytes alone. A recent research by Yuwei et al. showed that patient with severe COVID-19 tended to have a higher NLR, making it a mortality predictor for hospitalized COVID-19 patients.^{11,12}

Lymphocytes are mononuclear cells consisting of T-lymphocytes and B-lymphocytes both of, which are specific immune responses. The number of lymphocytes has been an interesting marker since COVID-19 was first identified. A study by Ian *et al.*, which conducted an analysis of the effect of characteristics of COVID-19 patients such as age and comorbidity to the lymphocyte count in COVID-19 patients showed a positive correlation between lymphopenia and the severity of COVID-19 in younger patients compared to older patients. In a study by Huang *et al.*, it was shown that COVID-19 patients requiring ICU care had lower Absolute Lymphocyte Count (ALC) and higher Lactate Dehydrogenase. Absolute lymphocyte count $< 0.6 \times 10^9/L$ is considered one of the early indicators for supportive care in the ICU. A research by Tan *et al.* also reported that lymphopenia was found in 51.39% of COVID-19

patients. A decrease in the number of lymphocytes implied a weakening of the adaptive immune system.¹³⁻¹⁵

According to the background, this study aimed to analyze NLR and ALC as predictor of the COVID-19 severity in patients.

METHODS

This study was a retrospective study with a cross-sectional method, which was carried out by taking secondary data from patients diagnosed with COVID-19 in the medical records of UNHAS Hospital and Makassar City Hospital from April to September 2020.

The study population was medical record data of patients aged ≥ 18 years diagnosed with COVID-19 by lung/internal medicine clinicians at the UNHAS Hospital and Makassar City Hospital. The study sample was an accessible population that met the inclusion criteria: patients with a diagnosis of COVID-19 who had data on the results of a Complete Blood Count (CBC) using Sysmex XS-800i and then classified into the non-severe COVID-19 criteria if symptoms of non-pneumonia or mild pneumonia were found and severe COVID-19 if visible symptoms including respiratory distress (shortness of breath) was found with a respiratory rate ≥ 30 times per minute, blood oxygen saturation ≤ 93 , PaO_2/FiO_2 ratio < 300 , and/or pulmonary infiltrates $> 50\%$ within 24-48 hours.

The samples were then statistically analyzed using SPSS version 22 and the Independent T-test. Meanwhile, the ability of NLR and ALC as predictors of severity was determined using a Receiver Operating Characteristics (ROC) curve analysis to obtain the cut-off value, p-value < 0.05 was considered as significant.

Approval of ethical eligibility was obtained from the Health Research Ethics Committee of Hasanuddin University Medical Faculty/Hasanuddin University Hospital/ Dr. Wahidin Sudirohusodo Hospital with number 472/UN4.6.4.5.31/PP36/2020.

RESULTS AND DISCUSSIONS

The study was performed at the Medical Record Installation of UNHAS Hospital and Makassar City Hospital during September 2020. A total of 393 patients was confirmed positive for COVID-19 wherein there were 17 patients aged < 18 years, resulting in a total of 376 subjects who met the inclusion criteria consisting of 327 non-severe COVID-19 patients and 49 severe COVID-19 patients.

Table 1. Characteristics of research subjects (n=376)

Variable	n	%
Gender		
Male	179	47.6
Female	197	52.4
Age		
< 30 years	87	23.1
30-49 years	191	50.8
>=50 years	98	26.1
Degree of severity		
Non-severe	327	87.0
Severe	49	13.0

Table 1 showed that most of research subjects were female (52.4%) and age ranging from 30 to 49 years (50.8%). Based on the degree of severity, most (327) subjects had non-severe COVID-19 (87.0%), while severe COVID-19 was found in 49 (13.0%) subjects.

Table 2 showed that the mean age of patients who participated in this study was 40.8 ± 14.5 years. This was in accordance with the research of Guan *et al.*, which showed that productive age had a quite high mobility and activity so it was suspected to be the cause of the spread of COVID-19.¹⁶ Meanwhile, the NLR varied between 0.14-44.81 with a mean of 4.78 ± 5.82 and ALC varied between $0.15 \times 10^3/\mu\text{L}$ - $5.26 \times 10^3/\mu\text{L}$ with a mean of $1.90 \pm 0.87 \times 10^3/\mu\text{L}$.

Neutrophils play a role in the innate immune response that results in organ failure and even death, whereas lymphocytes play a major role in the inflammatory response. Therefore, a high NLR indicates an imbalance in the inflammatory response

and is a marker of disease severity. This was in accordance with the results of this study, which can be seen in Table 3 showing a significantly higher NLR value in the severe group (9.81 ± 7.06) compared to the non-severe group (4.02 ± 5.22) ($p < 0.001$). It was shown that there was a significant relationship between NLR and the degree of disease, suggesting an accordance with the results of research by Liu *et al.*, and Jingyuan *et al.*^{12,17}

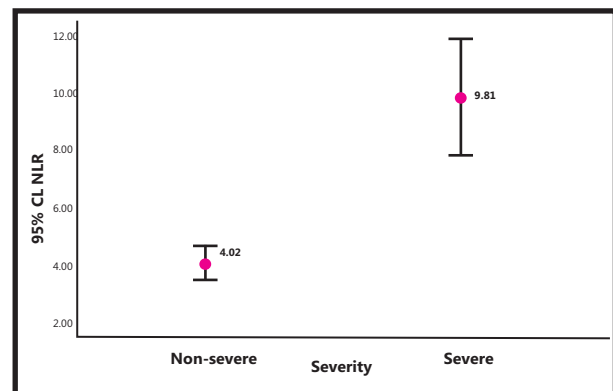
**Figure 1.** Comparison of NLR according to the severity of disease

Table 3 showed that the ALC was significantly lower in the severe group (1.22 ± 0.78) compared to the non-severe group (2.00 ± 0.83) ($p < 0.001$). It was suggested that there was a significant relationship between ALC and the degree of disease according to the results of a study by Huang *et al.*, which showed that about 85% of severe COVID-19 patients suffered from lymphopenia. Severe COVID-19 patients showed increased biomarkers of infection and inflammatory cytokines. The number of T-cells

Table 2. Mean age, neutrophils, lymphocytes, NLR, and ALC

Variable	Minimum	Maximum	Mean	SD
Age	18	88	40.84	14.53
Neutrophils	5.3	95.8	65.01	14.01
Lymphocytes	2.1	51.2	23.74	11.51
NLR	0.14	44.81	4.78	5.82
ALC	0.15	5.26	1.90	0.87

Table 3. NLR and ALC according to the severity of disease

Variable	Severity of Disease	n	Mean	SD	p
NLR	Non-severe	327	4.02	5.22	< 0.001
	Severe	49	9.81	7.06	
ALC ($\times 10^3/\mu\text{L}$)	Non-severe	327	2.00	0.83	< 0.001
	Severe	49	1.22	0.78	

decreased significantly in severe cases because T-helper cells and suppressor T-cells are reduced due to lymphocyte sequestration in certain target organs, wherein COVID-19 the target organ was the lungs.¹⁶ It was suggested that there was a significant relationship between ALC with the degree of disease, as in Figure 2.

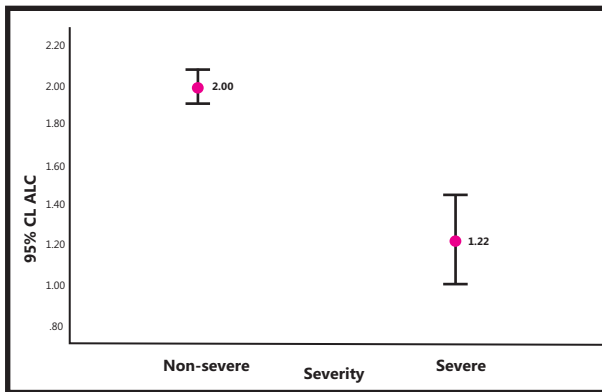


Figure 2. Comparison of ALC according to severity of the severity of disease

Receiver operating characteristics curve in Figure 3 showed that the Area Under Curve (AUC) for NLR was 0.856 ($p < 0.001$). It was suggested that NLR could be used as predictor marker of severity of COVID-19.

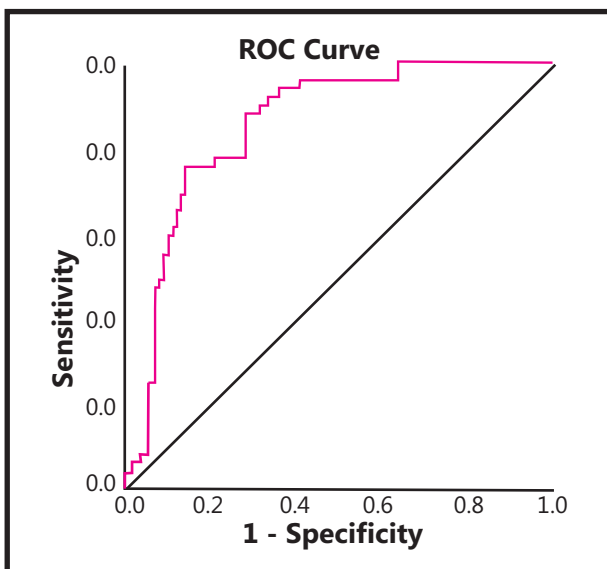


Figure 3. ROC curve of NLR according to the grade of severity of the disease

Based on the coordinates of the ROC curve, the NLR value with a cut-off value of 3.17 was considered to have an optimum sensitivity and specificity, with a value of 91.8% and 66.4%, respectively. In addition,

the positive likelihood ratio was $0.918/1-0.664=2.73$ and negative likelihood ratio was $1-0.918/0.664=0.12$.

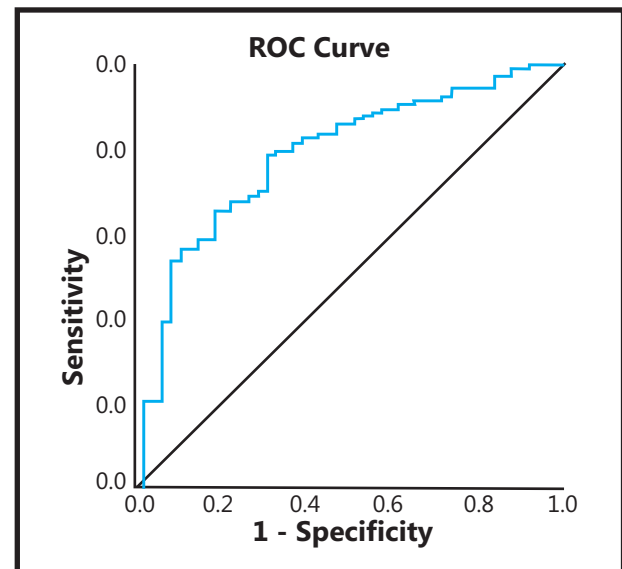


Figure 4. ROC curve of ALC according to severity of the disease

The AUC for ALC was 0.780 ($p < 0.001$), indicating that ALC could be used as a predictor of the severity of COVID-19.

The ROC curve of ALC with a cut-off point value of $1.74 \times 10^3/\mu\text{L}$ (Figure 4) was considered to have the optimum sensitivity and specificity, with value of 81.6% and 64.8%, respectively. In addition, the positive likelihood ratio was $0.816/1-0.648=2.31$ and negative likelihood ratio was $1-0.816/0.648=0.128$.

When the SARS-CoV-2 virus binds to the Angiotensin-Converting Enzyme 2 (ACE 2) receptor and enters the alveolar epithelial cells, the body responds through the body's innate immunity through its receptors including toll like receptors, which are expressed by increasing neutrophils and macrophages that play a role in phagocytosis and killing process of the pathogens together with the infected cells. Destruction of viruses and infected cells causes tissue damage that triggers the release of proinflammatory cytokines and expands inflammation resulting in increased NLR. In addition, T-lymphocytes also play a role in the body's immune response to SARS-CoV-2 infection. CD4 T-lymphocytes will activate B-lymphocytes to produce immunoglobulins, while CD8 T-lymphocytes will destroy virus-infected cells. Some studies suggested that a decrease in the number of peripheral lymphocytes (lymphopenia)

indicated the severity of COVID-19.¹⁸

The results of this study showed that the NLR and ALC could be used as predictors of the severity of COVID-19 through the analysis of the ROC curve with the AUC for NLR = 0.856 ($p < 0.001$) and ALC = 0.780 ($p < 0.001$). Based on the coordinate value of the ROC curve, the cut-off value of NLR was 3.17, giving a sensitivity of 91.8% and specificity of 66.4%. In addition, it was shown that the positive likelihood ratio = 2.73 (fair) and the negative likelihood ratio = 0.12 (excellent), indicating that the cut-off for NLR of 3.17 will provide sufficient probability in predicting the severity of COVID-19 in patients. This was in line with a prospective study by Liu *et al.*, which showed that the cut-off for NLR of 3.13 with a sensitivity of 87.5% and a specificity of 71.7% was used as an independent risk factor for COVID-19. Likewise, a research by Yang *et al.*, showed that the NLR cut-off of 3.3 with a sensitivity of 88% and a specificity of 63.6% was used as a prognostic factor for COVID-19 and to evaluate the severity of COVID-19 in patients.^{17,19}

Based on the results of this study, the cut-off value for ALC was $\leq 1.74 \times 10^3/\mu\text{L}$, giving a sensitivity of 81.6% and specificity of 64.8%. In addition, the positive likelihood ratio = 2.31 (fair) and negative likelihood ratio = 0.2 (very good), indicating that the ALC cut-off $1.74 \times 10^3/\mu\text{L}$ will provide sufficient probability in predicting the severity of COVID-19 of patients and its further classification into severe and non-severe. In accordance with a meta-analysis study by Huang *et al.*, the cut-off value used for ALC was $\leq 1.1 \times 10^3/\mu\text{L}$, which proved that lymphocyte levels were inversely proportional to the severity of COVID-19.¹³

The use of secondary data was one of limitations of this study, resulting in limited information from the medical records.

CONCLUSIONS AND SUGESSTIONS

The NLR value in severe COVID-19 patients was higher than that in non-severe COVID-19 patients. The cut-off for NLR ≥ 3.17 could be used as a predictor of COVID-19 with a sensitivity of 91.8% and a specificity of 66.4%. However, the ALC value was lower in severe COVID-19 patients compared to non-severe COVID-19 patients, and the cut-off for ALC $\leq 1.74 \times 10^3/\mu\text{L}$ could be used as a predictor for COVID-19 with a sensitivity of 81.6% and specificity of 64.8%. Researchers suggest conducting further research with a larger sample size.

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