

Relationship between Neutrophil-Lymphocyte Ratio and Disease Severity in COVID-19 Patients in Isolation Ward of Dr. Soetomo General Teaching Hospital

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Abstract

This study was conducted to prove the relationship between the neutrophil-lymphocyte ratio and the severity of COVID-19. A retrospective cohort study using medical record data of inpatients from June to July 2020. Analysis of the NLR relationship and the degree of severity using the Mann Whitney test if the data had an abnormal distribution. Significant if $p < 0.05$ and 95% confidence interval. If there were significant results, we try to measure the cut-off of NLR value to predict severe and non-severe clinical symptoms. Total study subjects were 110 patients, with a male as many as 65 (59.1%), the median age was 53.5 years (range 20-88). Most of the comorbidities were diabetes mellitus (35.5%), followed by hypertension (30%). The severity of clinical symptoms was 50% in the non-severe and severe groups, respectively. The NLR value was higher in the severe group. Mann-Whitney test showed significant differences in the value of NLR between the severe group and the not severe group with the p -value < 0.001 . Receiver operating characteristic (ROC) curve analysis area under the curve (AUC) of NLR on day-1 was 0.716 (CI 95%: 0.605 - 0.826), and the cut-off point of the prediction severity disease at day-7 was ≥ 6.14 with a sensitivity of 71% and a specificity of 69.1%. The neutrophil-lymphocyte ratio value with severe symptoms was higher than the neutrophil ratio value for lymphocytes with non-severe symptoms in patients with COVID-19.

Keywords: COVID-19, neutrophil lymphocytes ratio, risk-factors, clinical characteristics.

Introduction

COVID-19 is a contagious disease caused by SARS-CoV-2, a new type of coronavirus identified as the cause of a collection of pneumonia cases in the city of Wuhan, China, at the end of 2019. The virus is spreading rapidly, resulting in an epidemic

across China, followed by an increase in the number of cases worldwide^{1,2,3}. The number of fatalities due to COVID-19 continues to increase, and available evidence suggests the condition of some COVID-19 patients is rapidly deteriorating. Available therapy is supportive and may have limited effects with poor outcome^{4,5,6}. World Health Organization (WHO) reported 11.874.226 confirmed cases with 545.481 deaths worldwide (CFR 4.6%) until July 9, 2020. Indonesia reported its first cases on March 2, 2020, and until July 9, 2020, there were 70.736 confirmed

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cases COVID-19 with 3.417 cases died (CFR 4.8%)⁷. The rapid spread and serious dangers of COVID-19 require rapid analysis in identifying laboratory results and clinical characteristics with good precision and explaining the risk factors associated with mortality^{8,9}.

The neutrophil-lymphocyte ratio is a biomarker that can represent inflammation and immune status so that it can be indicators of the inflammatory response that are useful for assessing the prognosis of COVID-19 patients¹⁰. The neutrophil-lymphocyte ratio can be rapidly calculated based on routine blood tests performed at admission so that clinicians can make a precise and accurate disease assessment at an early stage, identify severe patients, and take active treatment measures as soon as possible to reduce mortality. This study was conducted to analyze the relationship between the neutrophil-lymphocyte ratio with the severity of symptoms of COVID-19 patients.

Materials and Methods

This research is an observational analytic study with a retrospective cohort study approach. Sampling was done by a consecutive sampling of the medical records of COVID-19 patients. The research sample

was COVID-19 patients who came to the hospital emergency room Dr. Soetomo General Hospital from June-July 2020. The inclusion criteria were patients aged ≥ 18 years diagnosed with COVID-19 with positive PCR swab results. The exclusion criteria included: patients with aplastic anemia, myeloproliferative disease, blood malignancy or chemotherapy, systemic lupus erythematosus (SLE) disease, patients with end-stage renal disease (ESRD). The severity of COVID-19 consists of no symptoms (asymptomatic), mild illness, moderate illness, severe illness, and critical illness. Patients with severe and critical symptoms were classified as severe. Patients who did not meet these symptoms were classified as non-severe. All data were analyzed with the statistical program SPSS version 25.0 (SPSS Inc, Chicago, USA).

Results and Discussion

There were 122 patients who met the inclusion criteria, and 12 patients were excluded so that 110 patients were eligible for the study. General characteristics of the subjects at admission to the hospital included age, gender, and comorbid (Table 1).

Table 1. General Characteristics of Research Subjects.

General Characteristics	n	%
Male	65	59.1
Female	45	40.9
Comorbid	76	69.1
Type 2 diabetes mellitus	39	35.5
Hypertension	33	30
Chronic kidney disease	17	15.5
Heart disease	12	10.9
HIV	4	3.6
Obesity	3	2.7
Malignancy	1	0.9
No Comorbid	34	30.9

Most of the COVID-19 hospitalized patients were male, with a total of 65 people (59.1%), while the female gender totaled 45 people (40.9%). 138 COVID-19 cases in 2020 in Wuhan showed 75 people (54.3%) were male and 63 (45.7%) female⁷. The Indonesian Health Ministry report until the end of June 2020 states that the percentage of COVID-19 in Indonesia in men is 51.5%, as well as a study conducted by the Chinese CDC which shows that most cases occur in men (51.4%). Most of the cases are found in the male sex^{3,8,9,10}.

In this study, T2DM was the most comorbid with 39 people (35,5%), followed by hypertension with 33 people (30%). Besides that, there were also 15,5% comorbid chronic kidney disease, 10,9% heart disease, and obesity 2,7%. 1482 cases in the United States as of the end of March 2020 showed some of the most common comorbidities such as

hypertension (49,7%), obesity (48.3%), chronic lung disease (34,6%), diabetes (28,3%) and cardiovascular disease (27,8%)¹¹. 5700 patients in New York in 2020 showed that the main comorbidities were hypertension (56,6%), obesity (41,7%), and diabetes (33,8%)¹². This could be due to the likely increase in ACE-2 receptor expression in hypertension, diabetes mellitus, and smokers⁸. Patients with these comorbidities also had poorer immune function¹³.

Respiratory symptoms become the most common symptoms because the transmission of SARS-CoV-2 in a human occurs mainly through respiratory droplets and infect the respiratory tract lining the alveoli⁸. Clinical symptoms such as fever (78,2 %), shortness of breath (76,4 %), and cough (75,5 %) were the most common symptoms in this study^{14,15}. Clinical and laboratory characteristics can be seen in Table 2 and Table 3.

Table 2 Clinical and Laboratory Characteristics.

Clinical and Laboratory Characteristics	N	%
Respiration symptoms		
Cough	83	75.5
Cold	31	28.2
Shortness of breath	84	76.4
Symptoms		
Fever	86	78.2
Anosmia	22	20
Gastrointestinal Symptoms		
Diarrhea	27	24.5
Nauseous vomit	43	39.1
Abdominal pain	23	20.9
Rapid Test		
Reactive	55	50
Non-reactive	55	50
CXR		

Cont... Table 2 Clinical and Laboratory Characteristics.

No abnormalities	13	11.8
Basal peripheral bilateral intercourse	53	48.2
Bilateral diffuse intercourse	25	22.7
Unilateral intercourse	11	10
Central bilateral intercourse	8	7.3
Thoracic CT scan		
Bilateral ground glass opacity	9	8.2
Unilateral ground glass opacity	4	3.6
No abnormalities	2	1.8
Not available	95	86.4

Table 3. Clinical and Laboratory Characteristics.

Clinical and Laboratory Characteristics	Result
Age (years), median (min - max)	53.5 (20 - 88)
Duration of symptoms before admission (days)	5.5 (1 - 14)
Median (min - max)	
Length of stay (days), median (min - max)	18 (1 - 58)
GCS	15 (8 - 15)
Vital signs, median (min - max)	
Systolic blood pressure (mmHg)	130 (90 - 170)
Diastolic blood pressure (mmHg)	80 (58 - 100)
Pulse (beat / minute)	103 (64 - 126)
Respiration rate (x / minute)	24 (16 - 36)
Temperature (o C)	37 (36.1 – 38.9)
SpO2 (%)	98 (96 - 99)
Laboratory, median (min - max)	
Hemoglobin (g / dl)	13.2 (3.9-17.2)

Cont... Table 3. Clinical and Laboratory Characteristics.

Leucocytes (/ mm ³)	8475 (1720 - 31010)
Platelets (/ mm ³)	236500 (18000 - 955000)
CRP (mg / dl)	9 (0.4 – 31.4)
Procalcitonin (ng / ml)	0.2 (0.01 - 14)
D-dimer (ng / ml)	490 (190 - 22730)
BUN (mg / dl)	15 (3 - 224)
Creatinine (mg / dl)	1 (0.1 – 18.5)
SGOT (U / L)	59 (12 - 293)
SGPT (U / L)	45 (12 - 263)
PO ₂ / FiO ₂	382.5 (23 - 895)

In this study, the median time to symptoms experienced by patients before admission to the hospital was 5.5 days. The median interval from symptom onset to hospital admission was seven days with a range of 3-9 days¹¹. A retrospective study by Wang et al. also showed the median interval from onset of symptoms to admission to hospital was 7 days with a range of 4-8 days⁷. This occurs because the symptoms of the second attack (pulmonary phase) occur four to seven days after the initial symptoms appear, which is characterized by the patient still having fever, starting hard to breathe, and the lung

lesions worsening^{8,16}.

The neutrophil-lymphocyte ratio on the first day had a median value of 5.6 (range 0.94-58.36). It was recorded that from 110 samples, the median neutrophils on the first day of examination was 6230 (range 1048-26110) with an increase in neutrophils in 70,9% of patients, while the median lymphocyte was 1070 (range 230-5360) with a decrease in lymphocytes in 82,7% of patients. Neutrophil levels, lymphocytes, and neutrophil-lymphocyte ratios on day-1 can be seen in Table 4.

Table 4. Neutrophil, Lymphocyte, and Neutrophil-Lymphocyte (NLR) Levels.

Absolute count of day 1	Result (µL)
Neutrophils, median (min-max)	6230 (1048-26110)
Lymphocytes, median (min-max)	1070 (180-5360)
Neutrophil lymphocytes ratio, median (min-max)	5.6 (0.94-58.36)

In 452 patients who showed a median neutrophil count of 3900 (range 2500-5800) with elevated neutrophils in 63.3% of patients with severe case symptoms and a median lymphocyte count of 900 (range 600 -1200) which is lower than the normal value¹⁷. 99 patients showed a median neutrophil count of 5000 (range 3300-8100) with showed an increase in 38% of patients and a decrease in the number of lymphocytes in 35% of patients ¹⁴. These results indicate a decrease in the absolute value of lymphocytes that corresponds to the second phase of attack (pulmonary phase) of the course of the disease.

The second attack phase occurred at a median of 4-7 days after the onset of initial symptoms^{8,16}, whereas the median time to symptoms experienced by the patient on admission was 5.5 days; in addition, the inflammatory system that began to occur in the second phase of attack significantly suppressed cellular immunity thereby reducing the number of lymphocytes^{4,16,18,19}. The severity level of clinical symptoms of COVID-19 was the same at admission, 55 cases (50%) in the non-severe (mild and moderate) group and in the severe (severe and critically ill) groups, respectively.

Table 5. Severity of Clinical Symptoms of COVID-19 Patients.

Severity Day 1	N	%
Non-severe	55	50
Mild	9	8.2
Moderate	46	41.8
Severe	55	50
Severe - Critically ill		

The comparison of the number of cases in the non-severe and severe groups was almost the same, namely from a total of 828 patients, 407 (49,15%) were in the severe group. This meta-analysis using the NLR and the ratio of lymphocyte-CRP (LCR) as a marker of inflammation which reflects systemic inflammation ²⁰. This could be because the data observed were data from patients who received treatment at the hospital. Generally, patients have entered the second phase of attack (pulmonary phase), where the hyper-inflammatory system response begins to occur in this phase.

The results of the Mann Whitney test showed that there is a significant difference in the value of the neutrophil-lymphocyte ratio on day 1 between the non-severe and severe groups on the 1st day (p<0.001) and 7th day (p=0.001) observations, however there was no significant difference in the value of the neutrophil-lymphocyte ratio on day 1 between the non-severe and severe groups at the end of observations (p=0.054). In this study, the median neutrophil-lymphocyte ratio on the first day of observation was 3.9 (range 0.95-58.36) in the non-severe group and 7.6 (range 0.94-48.06) in the severe group.

Table 6. Difference of Neutrophil-Lymphocyte Ratio Day 1 Based on Time of Observation.

Severity	N	Neutrophil-Lymphocyte Ratio Day 1	
		Median (min - max)	P value
Day 1			
Non-severe	55	3.9 (0.95 – 58.36)	<0.001
Severe	55	7.6 (0.94 – 48.06)	
Day 7			
Non-severe	55	4 (0.95 – 58.36)	0.001
Severe	31	8.2 (2.53 – 20.05)	
End			
Non-severe	77	5 (0.95 – 58.36)	0.054
Severe	33	6.8 (0.94 – 48.06)	

A total of 443 cases. The median NLR score in the severe group was 4.75 (2.51-9.42) higher than the median NLR for the non-severe group of 2.38 (1.57-3.72) with a p-value <0.001. A retrospective study conducted by Yang et al. on 93 cases also found an association between NLR values and the severity of COVID-19. The mean NLR value for the severe group was 20.7±24.1 higher than the mean NLR value for the non-severe group was 4.8 ± 3.5 with p-value <0.001. Meta-analysis conducted by Lagunas Rangel showed that NLR was significantly increased in COVID-19 patients with severe disease (SMD: 2.404, 95% CI: 0.98-3.82)²⁰.

The results of the Mann-Whitney test in this study showed a significant difference in the value of the neutrophil-lymphocyte ratio on the 1st day between the non-severe and severe groups on the 7th day of observation (p=0.001). 115 patients, it was found that the neutrophil-lymphocyte ratio was higher in

the severe group at admission than in the non-severe group (p = 0.0240 for cohort derivation and p <0.0001 for cohort validation) observed on day three and day seven. COVID-19 pneumonia is not severe in its early stages, but critically ill patients will worsen within 7-14 days of the disease and cause severe pneumonia and acute respiratory failure¹⁴.

Analysis of the ROC curve ratio of neutrophil-lymphocyte first day on severity observation of the seventh day showed the area under the curve (AUC) 0.716 (CI 95%: 0.605-0.826) and the best cut-off point of neutrophils lymphocytes ratio for predicting severe disease on day 7 were ≥ 6.14 with a sensitivity of 71% and a specificity of 69.1% (Figure 1). Extensive neutrophil-lymphocyte ratio AUC 0.841 and optimal cut-off point NLR to predict the poor clinical outcome is ≥ 3.3 with a sensitivity of 88% and a specificity of 63.6%⁴. The AUC area of the neutrophil-lymphocyte ratio was 0.737, and the optimal NLR cut-off value for

predicting the level of severe disease was ≥ 4.28 with a sensitivity of 56.3% and a specificity of 83.7%⁵.

Conclusion

In summary, there is a relationship between the neutrophil-lymphocyte ratio and the clinical severity of COVID-19 patients. The neutrophil-lymphocyte ratio can evaluate the level of clinical symptom severity, where the neutrophil-lymphocyte ratio value with severe symptoms is higher than the neutrophil-lymphocyte ratio value with non-severe symptoms in hospitalized COVID-19 patients.

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