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Diagnostic Value of Neutrophil To Lymphocyte Ratio For Assessing The Disease Severity In Covid-19 Patients

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ABSTRACT

Deterioration of the clinical condition in corona virus disease 2019 (Covid-19) patients can be rapid and unpredictable. A sensitive biomarker is required to assess the disease severity to anticipate these condition. Neutrophil to lymphocyte ratio (NLR) is easily calculated from a complete blood count parameters, so it can be used as a biomarker to assess disease severity in Covid-19 patients.

Patients characteristic and NLR were obtained from patients confirmed by Covid-19 at Udayana University Hospital, Bali, Indonesia from April to September 2020. The Mann Whitney U test is used to determine the difference of NLR in severe and non-severe cases of Covid-19. The receiver operating characteristic curve (ROC) is used to determine the optimal cut-off value, sensitivity, specificity, and area under curve (AUC) of the NLR in assessing disease severity in Covid-19 patients. Binary logistic regression analysis was performed to determine the effect of age, comorbidities and other significant variables that have a significant effect on disease severity.

A total of 411 patients were included in this study. We found a significant difference of NLR between severe and non-severe cases of Covid-19 ($p < 0.001$). We found an optimal NLR cut-off value of 3.0, with 81.4% sensitivity, 81.2% specificity, and area under curve of 0.886 (95% confidence interval (CI) 0.848 – 0.923; $p < 0.001$).

Neutrophil to lymphocyte ratio is a biomarker that has a high diagnostic value for assessing the disease severity in Covid-19 patients

Keywords: Biomarker, Covid-19, cut-off, neutrophil to lymphocyte ratio, severity

Introduction

Infection of the novel corona virus, known as severe acute respiratory syndrome corona virus 2 (SARS-Cov-2) has spread rapidly throughout the world. By the end of September 2020, more than thirty-six million people had been infected globally (1). Clinical symptoms can vary, from mild to severe and critical which can lead to death. Based on WHO interim guidelines, patients who are asymptomatic or have mild clinical symptoms, can perform self-isolation at home (2). However, healthcare professionals must be made aware of the worsening of clinical symptoms such as shortness of breath and acute respiratory distress syndrome (ARDS), which can occur on days 7 to 11 since the onset of symptoms (3). This is why a sensitive biomarker is required to assess the disease severity, so that the deterioration of the clinical condition can be anticipated.

Neutrophil and lymphocyte counts are obtained through a complete blood count examination, which is a routine examination done at the hospital. Approximately 7 to 14 days after the onset of symptoms, systemic inflammation occurs with the release of inflammatory mediators and cytokines known as cytokines storm (4). The inflammatory response can stimulate the production of neutrophils and lymphocytes apoptosis (5). This immune system dysregulation can be used as a marker of disease severity caused by a virus (6). A research in China done on 1.099 confirmed cases of Covid-19 found out that lymphopenia was more prominent in severe

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Table 1. Epidemiological characteristics and complete blood count parameters of Covid-19 patients

	Median (minimum-maximum)			
Variable	All patients (n = 411)	Non-severe (n = 313)	Severe (n = 98)	p value
Age, years	41 (19 – 82)	34 (19 – 75)	54 (21 – 82)	0.001
Sex				
Male (%)	262 (63.75)	198 (63.26)	64 (65.30)	0.713
Female (%)	149 (36.25)	115 (36.74)	34 (34.70)	
Comorbid				
Yes	86 (20.92)	25 (8.00)	61 (62.24)	< 0.001
No	325 (79.08)	288 (92.00)	37 (37.76)	
Hemoglobin, gr/dl	13.9 (7.9 – 17.1)	14.1 (8.9 – 17.1)	13.5 (7.9 – 17.1)	0.001
Leukocyte, x103 µL	6.88 (2.81 – 17.25)	6.65 (2.81 – 13.82)	7.61 (3.81 – 17.25)	< 0.001
Neutrophil, x103 µL	4.16 (1.30 – 15.50)	3.78 (1.30 – 12.72)	5.79 (2.56 – 15.50)	< 0.001
Lymphocyte, x103 µL	1.69 (0.31 – 5.92)	1.92 (0.66 – 5.92)	0.99 (0.31 – 5.70)	< 0.001
Monocyte, x103 µL	0.57 (0.05 – 1.66)	0.57 (0.23 – 1.66)	0.56 (0.05 – 1.64)	0.510
Platelet, x103 µL	241 (91 – 672)	231 (91 – 485)	270 (95 – 672)	0.012
NLR	2.3 (0.68 – 32.94)	1.93 (0.68 – 19.27)	5.88 (1.40 – 32.94)	< 0.001

compared to non-severe cases (7). Another study done on 155 Covid-19 patients found that there were higher neutrophil levels in refractory cases (8). Elevation of neutrophil counts and decreased of lymphocytes counts will be reflected in the NLR. This study aims to determine the differences of NLR in severe and non-severe cases, and determine the diagnostic value of NLR, so that it can be used as a simple biomarker for assessing the disease severity in Covid-19 patients.

Material and Methods

Study design and participants: This is a prospective study. Participants of this study were Covid-19 confirmed patients treated at Udayana University Hospital, Bali, Indonesia, which is a specialized hospital that treats Covid-19 patients. The diagnosis of Covid-19 were confirmed through a real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) examination of specimens obtained through nasopharyngeal swabs. The definition of severe case is based on World Health Organization (WHO) interim guidelines including fever or suspected respiratory infection plus one of the following: respiratory rate >30 breaths per minute; severe respiratory distress; or SpO2 ≤93% on room air (2). Patients under 18 years old, who are pregnant, and patients with hematologic disease are excluded from this study.

This study was approved by the Ethics Committee of Udayana University Hospital with the following approval number: 1010/UN14.2.2.VII.14/LT/ 2020, and has fulfilled the Helsinki Declaration.

Data Collection: Data is taken from the patient's medical records which includes epidemiological characteristics, clinical symptoms and signs, and laboratory tests including complete blood count. Laboratory tests are performed when the patient is admitted to the hospital.

Statistical Analysis: Data of all variables were analysed descriptively, continuous variables are presented in
the median and minimum – maximum, while categorical variables were presented as a percentage. We divided the patients into two categories, severe and non-severe Covid-19 based on WHO interim guideline. Mann Whitney U test were used to determine differences in numerical variables, while Chi-square test were used to determine differences in categorical variables. The ROC method was used to determine the optimal cut-off value, sensitivity, specificity, and area under curve of the NLR in assessing disease severity. Variables that have a significant effect on disease severity are transformed into dichotomous variables. Binary logistic regression analysis was performed to determine the effect of age, comorbidities and other significant variables. The result was considered statistically significant if was p <0.05. Statistical calculations were done using SPSS 25.0 software.

Results

Epidemiological characteristics and complete blood count parameters: A total of 411 patients were included in this study. Table 1 shows the

Table 2. Diagnostic values of leukocyte, absolute neutrophil counts, and NLR

Variable	Cut-off value	Sensitivity (%)	Specificity (%)	Area under curve	95% Confidence Interval	p value
Leukocyte, x103 µL	> 7.0	60.8	54.1	0.624	0.557 – 0.692	< 0.001
Neutrophil, x103 µL	> 4.2	77.3	60.2	0.759	0.704 – 0.814	< 0.001
NLR	> 3.0	81.4	81.2	0.886	0.848 – 0.923	< 0.001

Table 3. The OR and adjusted OR of leukocyte, neutrophil counts, and NLR

Variable	Crude odds ratio (95% CI)	p	Adjusted odds ratio (95% CI)	P value
Leukocyte	1.833 (1.152 – 2.916)	0.011	0.846 (0.316 – 2.263)	0.738
Neutrophil	4.599 (2.752 – 7.686)	< 0,001	1.784 (0.850 – 3.745)	0.126
NLR	18.969 (10.568 – 34.049)	< 0.01	9.073 (4.717 – 17.453)	< 0.001

epidemiological characteristics and complete blood count parameters of the study subjects of this study. A total of 98 patients (23.84%) are severe patients, while 313 patients (76.16%) are non-severe patients. There are significant differences in age, comorbid, hemoglobin, leukocytes, absolute neutrophils, lymphocytes and platelet count, and NLR in severe compared with non-severe patients. However, there are no significant differences in sex and absolute monocyte count.

Optimal cut-off values, sensitivity, specificity, and area under curve: Using ROC analysis, we look for the optimal cut-off value and the area under curve of each variable (Figure 1 and Table 2). The optimal cut-off values of leukocyte, absolute neutrophil count, and NLR are 7.0 x10^3 µL; 4.2 x10^3 µL; and 3.0 respectively. NLR has the highest sensitivity, specificity, and area under the curve.

Association between NLR and the risk of severe Covid-19: To determine the factors that influence the occurrence of severe Covid-19, we conducted a logistic regression analysis to obtain a crude odds ratio (OR). We made adjustments for the age and comorbid variables because they affected the occurrence of severe Covid-19 to obtain the adjusted odds ratio. The results show that NLR still has a significant effect on the occurrence of severe Covid-19. However, there was no significant effect of leukocyte and neutrophil counts after adjustment (Table 3).

Discussion

The result of this study shows that absolute lymphocyte count in severe cases of Covid-19 is significantly lower compared to non-severe cases. These results are consistent with studies by Sun et al who found peripheral blood cell abnormalities in Covid-19 patients, especially a decrease of lymphocyte counts (9). The immune response to the virus depends on lymphocyte function. Systemic inflammation can suppress the cellular immunity as a result of decreased CD4+ lymphocyte counts (10). Low lymphocyte counts can cause disruption of the immune system, making bacterial infections more easily occur in severe Covid-19 patients (11). In this study we also found a significantly higher absolute neutrophil count in severe compared to non-severe patients. Neutrophils can interact with other cells to produce cytokines which can cause tissue damage (12). Neutrophils can be induced by inflammatory factors such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 which is produced by lymphocytes (13).

In this study, the NLR in severe cases was significantly higher compared to non-severe cases. Neutrophil lymphocyte ratio also indicates the inflammatory status of the patient (14). This shows the occurrence of a systemic inflammatory reaction that triggers a cytokine storm that can cause tissue damage (8), thus NLR can be used as a biomarker to assess the disease severity in Covid-19 patients. Liu et al has found that the increase in NLR is a biomarker to a poor clinical outcome in patients Covid-19 (15). NLR is also used as a biomarker to assess the severity of bacterial infections (16). Bacterial infections play an important role in poor outcomes in Covid-19 patients, so adequate antibiotics are needed, especially in severe cases (9). In addition to viral infections, the NLR has also been used to assess the diseases severity in malignancy (17), bacterial

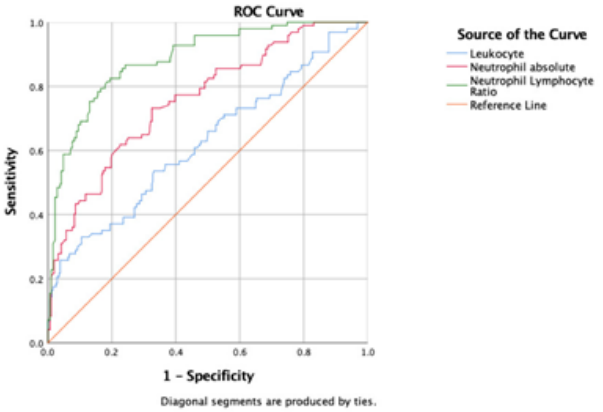

Fig. 1. The ROC analysis to determine the diagnostic value of leukocyte, absolute neutrophil counts and NLR for assessing the disease severity in Covid-19 patients

pneumonia (18), autoimmune diseases (19) and tuberculosis (20).

In this study we found a high sensitivity, specificity, and area under curve of the NLR to assess the disease severity in Covid-19. These results are consistent with study of Yang et al which found an area under curve of NLR of 0.841, with sensitivity and specificity of 88.0% and 63.6%, respectively (21). The optimal cut-off value of NLR that is found in this study is 3.0 x10^3 µL. Sun et al and Yang et al have found the optimal cut-off values for the NLR of 3.3 and 4.5, respectively (9,21). Another study by Yang et al have found that in patients with NLR ≥3.3 and age >49.5 years old, as many as 46.1% of patients with mild clinical symptoms will become severe (21). Neutrophil lymphocyte ratio in assessing the disease severity has several advantages, including easily calculated from parameters of complete blood count, can be available in all hospitals, and can provide results in a short time. These advantages can help the clinicians in increasing of awareness and decision making in the management of Covid-19 patients.

There are several limitations in this study. Firstly, the data obtained are from a single research center. Second, the lack of clinical data of patients that can affect the parameters of complete blood count.

The NLR is a biomarker that has a high diagnostic value in assessing the disease severity in Covid-19 patients. Monitoring of complete blood count parameters is important for assessing the disease progression.

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