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Comparison of Neutrophil-Lymphocyte Ratio in Patients with COVID-19 and Dengue Hemorrhagic Fever

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ABSTRACT

Coronavirus Disease-19 (COVID-19) and Dengue Hemorrhagic Fever (DHF) can be found together in patients and are difficult to distinguish because they have the same antigenic viral structure, symptoms, and laboratory findings. Neutrophil-Lymphocyte Ratio (NLR) is one of the inflammatory markers that are both easy and have fast results, so it is considered adequate for managing both diseases. This study aimed to compare NLR values and cut-off based on degrees in COVID-19 and DHF patients. The study used data from medical records of 459 COVID-19 patients and 95 DHF patients treated at UNHAS RSPTN and Sayang Rakyat Hospital from July 2020 to August 2021. They had routine hematological examination results when they were first admitted. COVID-19 samples were grouped into severe and moderate degrees, and DHF samples were grouped into DHF without shock and DHF in shock. Mann-Whitney test, independent T-test, and ROC curve were used to compare the two groups (significant if the p-value was <0.05). There was a difference in NLR median between moderate COVID-19 (2.57) and severe COVID-19 (6.39) (p<0.001); between DHF without-shock (0.49) and DHF in-shock (0.43) (p<0.001). Neutrophil lymphocyte ratio cut-off between moderate and severe COVID-19 based on ROC curve was 5.66 (sensitivity 79.6%, specificity 73.5%), and between DHF without-shock and DHF in-shock was 0.39 (sensitivity 93.2%, specificity 83.3%). Neutrophil lymphocyte ratio values in COVID-19 are different from DHF. Neutrophil-lymphocyte ratio values are higher in severe COVID-19 than moderate, while NLR values are lower in DHF in shock rather than without shock.

Keywords: COVID-19, dengue hemorrhagic fever, neutrophil-lymphocyte ratio

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease that is caused by a virus from the Coronavirus group, namely Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which WHO designated as a pandemic on March 12, 2020. COVID-19 can be divided by degree of severity: asymptomatic, mild, moderate, severe, and critical. Data from WHO states that on August 9, 2022, there were 583,038,110 positive cases of COVID-19 worldwide, with a death count of 6,416,023. In Indonesia, it was reported on August 10, 2022, there were 6,216,621 confirmed cases of COVID-19, with 6,010,545 recovered and 157,028 died (CFR 2.5%). In South Sulawesi, there were 143.971 confirmed cases, with a mortality rate of 2,481 cases.[1–3]

Along with this problem, in tropical countries, Dengue Hemorrhagic Fever (DHF) is caused by one of the four Dengue viruses (DENV-1,-2,-3,-4), which are transmitted from the bite of the female *Aedes aegypti* mosquito infected by the dengue virus, is an ongoing public health problem. The World Health Organization (WHO) divides dengue fever into four degrees. Degrees I and II are called DHF without shock, and degrees III and IV are called DHF with shock or Dengue Shock Syndrome (DSS). In Indonesia, reported cases of dengue fever as of December 2020, there were 95,971 positive cases of dengue fever with 663 deaths.[4,5]

The existence of COVID-19 at the same time as dengue fever during the COVID-19 pandemic, especially in dengue-endemic areas such as Indonesia, is often found and causes errors during diagnosis due to the similarity in the structure of viral antigens, laboratory results, and clinical manifestations, which are almost the same, making them difficult to differentiate. One of the easiest to check, relatively more cost-efficient, and quick to apply daily inflammation and infection markers can be used for managing these two diseases, namely the Neutrophil-Lymphocyte Ratio (NLR). The NLR is

the ratio of the absolute number of neutrophils and the absolute number of lymphocytes obtained from the differential leukocyte count, a component of the Complete Blood Count (CBC) examination. Neutrophils play an essential role in the innate immune response, while lymphocytes are important in the adaptive immune response. The combination of changes occurring in these two immune system pathways is an excellent indicator for assessing systemic immune complex responses. This theory is the basis for the importance of NLR examination, rather than just neutrophils or lymphocytes alone.[6,7]

Several studies say that the NLR value is significantly higher in COVID-19 patients with severe clinical conditions due to an increase in the number of neutrophils and a decrease in the number of lymphocytes. This result is due to Angiotensin-Converting Enzyme 2 (ACE2) as the primary receptor for SARS-CoV-2, which can be expressed on lymphocytes, causing SARS-CoV-2 to directly infect these cells, causing lymphopenia. It makes the body vulnerable to bacterial invasion and increases neutrophils. In addition, proinflammatory cytokines such as IL-10, IL-6, and Tumor Necrosis Factor (TNF)- can activate neutrophils and damage lymphocytes. Meanwhile, in DHF, the lower the NLR value, the greater the severity of the DHF infection because in the critical phase of DHF, there is a decrease in the number of neutrophils and an increase in the number of lymphocytes due to the regulation of neutrophil apoptosis by viral infection.[8–10]

Based on this background, researchers are interested in comparing NLR values and cut-offs based on degrees in COVID-19 and dengue fever patients to help clinicians rapidly manage these two diseases.

METHODS

A retrospective study with a cross-sectional method used secondary data from 459 COVID-19 patients and 95 dengue fever patients who were diagnosed based on history, physical examination, and laboratory examination by clinicians at the Pulmonology/Internal Medicine Medical Staff at Hasanuddin University Hospital and Sayang Rakyat Hospital Makassar, during July 2020 until August 2021. The research sample was the accessible population meeting the inclusion criteria, namely data on patients diagnosed with COVID-19 and dengue fever who were ≥ 18 years old and had data on the results of a CBC examination at the time of initial admission to the hospital. Data of patients with infectious diseases caused by bacteria, coronary heart disease, malignancy, autoimmune disease, or incomplete medical record data were excluded from this study. COVID-19 samples were grouped into moderate and severe degrees, and DHF samples were grouped into no shock and shock based on WHO.

Data analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 22. The statistical methods used were frequency distribution calculations and statistical tests, data normality tests using Kolmogorov-Smirnov. The statistical test used to test continuous variables that were normally distributed was the independent T-test, while those not normally distributed used the Mann-Whitney test. Determination of the NLR cut-off was based on the Receiver Operating Characteristics (ROC) curve. The results were significant if the p-value <0.05. Ethical approval was obtained from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Hasanuddin University (FKUH)/Hasanuddin University Hospital (RSUH)/Dr Wahidin Sudirohusodo Hospital, with article number 554/UN4.6.4.5.31/PP36/2021.

RESULTS AND DISCUSSIONS

This study showed that of the 459 COVID-19 samples, the majority were male (52.1%), while of the 95 dengue fever patient samples, the majority were female (51.6%). The age range in the COVID-19 group was 20-80 years old, and in the DHF group was 18-79 years old. Most patients were 56-65 years old (22.7%) in the COVID-19 group, and 18-25 years old (41.1 %) in the DHF group (Table 1).

Based on the data normality test using the Kolmogorov-Smirnov test, neutrophil and lymphocyte data in the COVID-19 and DHF groups

Table 1. Sample characteristics of the COVID-19 and DHF groups

Criteria	DHF (n/%)	COVID-19 (n/%)
Gender		
Male	46 (48.4 %)	239 (52.1 %)
Female	49 (51.6 %)	220 (47.9 %)
Age (years old)	29 (18-79)	51 (20-80)
18-25	39 (41.1 %)	37 (8.1 %)
26-35	22 (23.2 %)	95 (20.7 %)
36-45	10 (10.5 %)	52 (9.4 %)
46-55	15 (15.8 %)	80 (17.4 %)
56-65	5 (5.3 %)	104 (22.7 %)
>65	4 (4.2 %)	91 (19.8 %)

were normally distributed with a p=0.200 (p>0.05), so the independent T-test was used. Meanwhile, the NLR data in the COVID-19 and DHF groups were not normally distributed with a p<0.001 (p<0.05), so the Mann-Whitney test was used.

The neutrophil mean in moderate COVID-19 was lower (61.9 cells/L) than in severe COVID-19 (79.22 cells/L), while the lymphocyte mean in moderate COVID-19 was higher (25.66 cells/L) compared to the severe (13.77 cells/L), and the median NLR in moderate COVID-19 was lower (2.57) than in severe (6.39). The Mann-Whitney test for the median neutrophils, lymphocytes, and NLR found significant differences between moderate and severe degrees of COVID-19 with p-values p<0.001; p<0.001; p<0.001, respectively (Table 2).

Mean neutrophils in DHF without shock were higher (28.06 cells/L) compared to DHF with shock (25.72 cells/L), while the mean lymphocytes in DHF without shock were lower (56 cells/L) compared to DHF with shock (60 .82 cells/L), the median NLR in DHF without shock was higher (0.49) compared to shock (0.43). The independent T-test for mean neutrophils and lymphocytes and the Mann-Whitney test for median NLR showed significant differences between DHF without shock and with shock, with p-values being p<0.001; p<0.001; p<0.001, respectively (Table 3).

The independent T-test showed a significant difference in the number of neutrophils and lymphocytes between COVID-19 and DHF (p<0.05). Mean neutrophils showed higher values in COVID-19 than in DHF (62.6 vs. 27.9 cells/L), and mean lymphocytes were found to be higher in DHF than in COVID-19 (56.2 vs. 25.1 cells/L). The Mann-Whitney test showed a significant difference in the NLR value between COVID-19 and DHF (p<0.05). Median NLR showed a higher value in COVID-19 than in DHF (2.6 vs. 0.46), and there was a significant difference between the two groups (p<0.05) (Table 4).

The ROC curve for COVID-19 shows that the Area Under Curve (AUC) was 0.828 (82.8%) and was significant (p<0.001), the NLR cut-off point value was 5.66, giving optimal sensitivity and specificity values, namely sensitivity 79.6% and a specificity of 73.5% to distinguish between moderate and severe degrees of COVID-19. Apart from that, the ROC curve for DHF showed that the AUC was 0.906 (90.6%) and was 0.39, giving optimal sensitivity and specificity values, namely sensitivity of 93.2% and a specificity of 83.3% to differentiate between DHF without shock and with shock (Figure 1).

The sample characteristics in this study show that most COVID-19 patients were male, while most dengue fever patients were female. Meta-analysis research conducted by Abate *et al.* states that the prevalence of COVID-19 occurs more in males than females. Data from the Indonesian Ministry of Health shows that the percentage of males and females

Table 2. The differences in NLR according to COVID-19 severity

Variable	COVID-19 Severity		p
	Moderate	Severe	
Neutrophils (10^3 cell/μL) (mean±SD)	61.9±14.74	79.22±9.59	< 0.001*
Lymphocytes (10^3 cell/μL) (mean±SD)	25.66±12.55	13.77±5.36	< 0.001*
NLR (median (min-max))	2.57 (0.02-14.37)	6.39 (3.18-10.49)	< 0.001**

* Independent T-test; **Mann-Whitney test

Table 3. Differences in NLR according to DHF stage

Variable	DHF Stage		p
	Without Shock	Shock	
Neutrophils (10^3 cell/μL) (mean±SD)	28.06±10.8	25.72±4.45	< 0.001*
Lymphocyte (10^3 cell/μL) (mean±SD)	56±12.65	60.82±10.17	< 0.001*
NLR (median (min-max))	0.49 (0.08–1.99)	0.43 (0.37–0.46)	< 0.001**

* Independent T-test; **Mann-Whitney test

Table 4. The difference between NLR in COVID-19 and DHF groups

Variable	DHF	COVID-19	p
Neutrophils (10^3 cell/μL) (mean±SD)	27.9±10.6	62.6±14.9	0.008*
Lymphocyte (10^3 cell/μL) (mean±SD)	56.2±12.5	25.1±12.5	< 0.001*
NLR median (min-max))	0.46 (0.08–1.99)	2.6 (0.02–14.37)	< 0.001**

* Independent T-test; **Mann-Whitney test

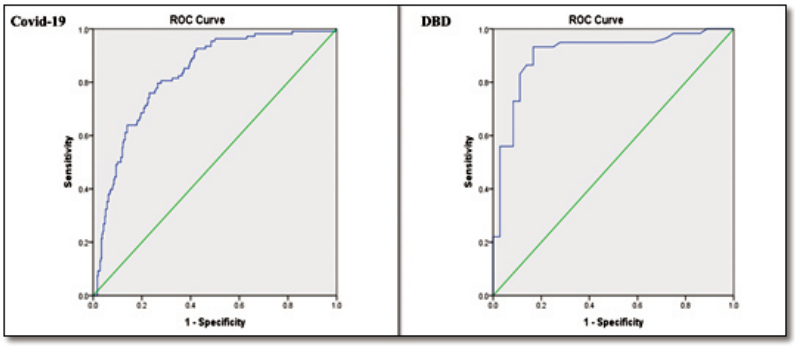

Figure 1. ROC curve of NLR in COVID-19 and DHF

infected with DHF tends to be the same. Based on age group, most were found at ages 56-65 for COVID-19 and ages 18-25 years for DHF. This finding is in line with previous research conducted by Rosso *et al*., which showed that dengue fever patients were significantly younger than COVID-19 patients.[11–13]

The number of COVID-19 neutrophils in the moderate group was lower than in the severe (p<0.001), following research by Li *et al*. that in severe COVID-19 patients, the neutrophil count gradually increased. Meanwhile, the number of lymphocytes for moderate COVID-19 was higher than for severe (p<0.001), in line with research by Huang *et al*., which stated that most COVID-19 patients with severe clinical degrees experienced lymphopenia. The NLR value in moderate COVID-19 was lower than in severe COVID-19 (p<0.001). This study's results align with previous studies by Ding *et al*., Li *et al*., and Alkhatip *et al*., which stated that NLR was significantly higher in COVID-19 patients with severe symptoms compared with patients with non-severe symptoms. This research obtained an NLR cut-off value of 5.66 (sensitivity 79.6%; specificity 73.5%), which is used as the limit between moderate and severe degrees of COVID-19.[14–17]

Dengue hemorrhagic fever without shock had a higher neutrophil count than those with shock (p<0.001), following research by Yanti *et al*. that patients with neutropenia were found in the DHF group with severe clinical symptoms. The number of DHF lymphocytes without shock was lower than with shock (p<0.001), in line with research conducted by Chastity *et al*., which stated that the more the lymphocytes increased, the higher the clinical grade. The NLR value in DHF without shock was higher than in DHF patients in shock (p<0.001). This study's results align with previous research by Yuditya *et al*. that the lower the NLR value, the greater the severity of dengue fever infection. This study obtained an NLR cut-off value of 0.39 (sensitivity 93.2%; specificity 83.3%), which was used as the limit between no shock and shock in DHF.[9,18,19]

The number of COVID-19 neutrophils was higher than that of DHF (p=0.008), while the number of lymphocytes of COVID-19 was lower than that of DHF (p<0.001), and the NLR value of COVID-19 was higher than that of DHF (p<0.001). This study is in line with the results of a cross-sectional study conducted by Rosso *et al*., which showed that in COVID-19 patients an increase in the number of neutrophils and a decrease in the number of lymphocytes, while in DHF patients, there was a decrease in the number of neutrophils and an increase in the number of lymphocytes resulting in a higher NLR value in COVID-19 compared to DHF (p<0.001).[13]

A gradually decreasing lymphocyte count can be found in patients with severe or non-surviving COVID-19, while the neutrophil count gradually increases. This result is because when the SARS-Cov-2 virus binds to the ACE2 receptor and enters the alveolar epithelial cells, the body will respond through the body's innate immunity defense system through its receptors, including Toll-Like Receptors (TLR), macrophages carry out phagocytosis and destroy pathogens with the infected cells. Destruction of viruses and infected cells causes tissue damage, triggering the release of proinflammatory cytokines such as IL-6, IL-10, and TNF-α, which will cause neutrophil activation, causing neutrophilia. Increased lymphocyte apoptosis can lead to adaptive immune system immunosuppression, which will trigger further systemic inflammatory reactions, as well as the risk of developing nosocomial infections and opportunistic bacteria, thus triggering an increase in neutrophils also causing neutrophilia. The number of lymphocytes decreases gradually in COVID-19 patients because ACE2 has been considered the main receptor for SARS-CoV-2, which can be expressed in lymphocytes, causing SARS-CoV-2 to directly infect these cells and eventually cause lymphopenia. Cytokines such as IL-10, IL-6, and TNF-α can damage lymphocytes, also causing

lymphopenia in COVID-19 patients. Several studies say that a decrease in the number of lymphocytes in the peripheral blood indicates the severity of COVID-19.[8,20]

Dengue hemorrhagic fever can cause a decrease in the number of leukocytes and neutrophils, along with relative lymphocytosis that occurs during fever. Changes in NLR values (neutrophils<lymphocytes) help predict the critical period due to plasma infiltration. This neutropenic state is caused by suppression of the bone marrow due to the process of viral infection or an indirect mechanism through the production of proinflammatory cytokines, which induce apoptosis in neutrophils. The apoptosis process begins when the dengue virus enters target cells, causing the cells to release cytochrome-c. Cytochrome-c via Apaf-1 will activate inactive procaspase-9 into active caspase-9. Active caspase-9 will activate inactive procaspase-3 into active caspase-3. This active caspase-3 will stimulate apoptosis. The more severe the apoptotic process, the more severe the disease. This mechanism supports the protective role of neutrophils in the antiviral response. In addition, relative lymphocytosis in the presence of atypical lymphocytosis is a consistent finding in DHF cases. This increase in lymphocytes occurs due to the mechanism of the immunopathogenesis of dengue virus infection, which involves a humoral response in the form of antibody formation, which plays a role in the process of virus neutralization, complement-mediated cytolysis, and antibody-mediated cytotoxicity, also involving T lymphocytes, both T-helper (CD4) and T cytotoxic (CD8), monocytes and macrophages, cytokines and complement activation.[10,21]

This study has limitations in the form of a CBC examination, which was only carried out at the initial admission of the patient being treated, so it cannot be observed serially, and the use of secondary data, causing the information to be limited to what was written from the medical record.

CONCLUSIONS AND SUGGESTIONS

The NLR value in COVID-19 was different from DHF. The NLR was higher in severe COVID-19 compared to moderate COVID-19, while NLR was lower in DHF patients in shock than without shock. An NLR > 5.66 can detect severe COVID-19 with a 79.6% sensitivity and 73.5% specificity, while an NLR < 0.39 can detect in-shock DHF with a 93.2% sensitivity and 83.3% specificity.

This research is suggested to be the basis for clinicians in differentiating COVID-19 and DHF and for further research using a proportional sample comparison between COVID-19 and DHF.

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