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# The Correlation Between Neutrophil Lymphocyte Ratio And Length Of Stay Of COVID-19 Patients At Dr. Zainoel Abidin General Public Hospital Banda Aceh



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## ABSTRACT

**Introduction:** Coronavirus disease 2019 or COVID-19 infection has showed an immunologic dysregulation that could affect the clinical outcomes. Neutrophil lymphocyte ratio or NLR is one of inflammation marker that rapid and easily available at the initial moment of hospitalization. This study aimed to determine the correlation between NLR and length of stay of COVID-19 patients at Dr. Zainoel Abidin General Public Hospital Banda Aceh.

**Methods:** This was an analytic retrospective study that observed medical records and complete blood count data of 577 COVID-19 hospitalized patients on January – March 2022. The sample of this study was elected by purposive sampling and the sample size was 83 that obtained by lemeshow formula (1990).

**Result:** The results showed that COVID-19 was dominated by male (53%) and 26 – 35 age group (19.3%). The value of NLR was dominated by  $\geq 3.13$  group (60.2%). The length of stay was dominated by  $\leq 11$  days group (69.9%). Bivariate analysis of this study was Spearman Rank test. The results of correlation test obtained p-value was 0.003 and r was 0.319.

**Conclusion:** This study obtained that was a significant correlation between NLR and length of stay of COVID-19 patients at Dr. Zainoel Abidin General Public Hospital Banda Aceh.

**Keywords:** Coronavirus disease 2019, neutrophil lymphocyte ratio, length of stay.

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## INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly virulent infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. The first COVID-19 infection was identified in China in late 2019 and officially declared as pandemic by the World Health Organization (WHO) in March 2020.<sup>1</sup> The COVID-19 pandemic has had a major impact on Indonesia, the fourth largest country by population. The number of confirmed positive COVID-19 cases in Indonesia reached over 1.5 million by April 2021, consequently ranking Indonesia among the states with the highest prevalence of confirmed cases in Asia and globally.<sup>2</sup>

Laboratory findings in COVID-19 cases indicate lymphocytopenia accompanied by leukocytosis thereby an increased neutrophil lymphocyte ratio (NLR).<sup>3</sup>

Research by Mehta et al also showed that in severe COVID-19 patients, there is an underlying hyperinflammatory response that leads to a cytokine storm, resulting in multi-organ failure and death.<sup>4</sup> Increased inflammatory response, indicated by neutrophilia and lymphocytopenia, can be found in patients with clinical deterioration. These changes in laboratory values can be demonstrated by changes in the patient's NLR value.<sup>5</sup> Research conducted by Selanno et al. showed that patients with severe COVID-19 had a mean NRL value of 9.81 compared to patients with non-severe COVID-19, which was 4.02 ( $p < 0.001$ ).<sup>6</sup>

The data and description above indicate a tendency for differences in prognosis that can be found in COVID-19 patients. Research by Pramana et al. suggests that NRL examination should be performed earlier in COVID-19 cases because it is considered as a simple, easy, and

effective test in screening management of COVID-19 patients.<sup>7</sup> The data of length of stay obtained from patient medical records can be used as one of the factors that could be assessed for its potential contribution to improving the clinical outcome of COVID-19 cases. Research on NLR associated to length of stay in COVID-19 case has not been conducted at Dr. Zainoel Abidin General Public Hospital, which serves as the referral hospital in Aceh Province. This prompted the researcher to conduct a study on the correlation between the neutrophil lymphocyte ratio and the length of stay of COVID-19 patients at RSUDZA Banda Aceh.

The objective of this study is to analyze the correlation between the neutrophil lymphocyte ratio and length of hospital stay in COVID-19 patients at Dr. Zainoel Abidin General Public Hospital Banda Aceh.

## METHODS

This study was an analytical observational study using retrospective data. Data was taken from the medical records of COVID-19 patients at Dr. Zainoel Abidin General Public Hospital Banda Aceh. The study population consisted of all patients who met the criteria for COVID-19, were hospitalized, and underwent blood tests at Dr. Zainoel Abidin General Public Hospital during January to March 2022.

The sample for this study consists of hospitalized COVID-19 patients who underwent blood tests at Dr. Zainoel Abidin General Public Hospital Banda Aceh during January to March 2022 and met the inclusion and exclusion criteria. The inclusion criteria for this research sample are: (1) Patients confirmed with COVID-19 and positive PCR, (2) Patients admitted to the COVID-19 isolation ward at Dr. Zainoel Abidin General Public Hospital during January to March 2022, (3) Patients who underwent a complete blood count during their hospitalization at Dr. Zainoel Abidin General Public Hospital.

The exclusion criteria for the sample are: (1) Patients with incomplete medical records, (2) Patients with clinical symptoms of COVID-19 but negative PCR results, and (3) Patients with mortality outcome when discharged. The sampling technique used in this study is non-probability sampling using the purposive sampling method. The sample size used in this study was obtained using the formula for known population size, namely the Lemeshow formula (1990). The population size was known to be 577 subjects. The sample size was obtained by substituting the population size variable values into the Lemeshow formula, resulting in a sample size of 83 subjects.

This study has received ethical approval from the Health Research Ethics Committee of Dr. Zainoel Abidin General Public Hospital in October 2022. Data were obtained from the Medical Records Unit and Clinical Pathology Laboratory of Dr. Zainoel Abidin General Public Hospital from November to December 2022.

This study used univariate and bivariate analysis. Results derived from the univariate analysis demonstrate the

distribution, frequency, and percentage of the studied variables, which will be presented in table form. The univariate data are general characteristics, NRL, and length of stay. Bivariate analysis was used to examine the relationship between the independent variable, NRL value, and the dependent variable, length of stay for COVID-19 patients. The relationship in this study was proven using the Spearman Rank test.

## RESULTS

This study was conducted from November to December 2022 at the Medical Records Center and Clinical Pathology Laboratory of Dr. Zainoel Abidin General Public Hospital Banda Aceh. This study used secondary data found in the medical records of 83 subjects who meet the inclusion and exclusion criteria. The characteristics of the subjects are grouped based on the age and gender of the subjects. The general characteristics of the research subjects are shown in **Table 1**.

The results, presented in **Table 1**, indicate that the distribution of research subjects by gender is dominated by males.

The number of subject in the male group is 44 subjects (53%), while the female group has 39 subjects (47%). The results shows that the highest number of research subjects is found in the 26–35 age group, with 16 subjects (19.3%). The lowest number of research subjects is found in the 5–11 age group, with 1 subject (1.2%).

The characteristics of the NRL in COVID-19 patients who were the subjects of the study are shown in **Table 2**.

The results, presented in **Table 2**, shows the neutrophil-lymphocyte ratio values of subjects with a cut-off value of 3.13. The majority of research subjects were found in the high-risk group with 50 subjects (60.2%). The number of subjects with low risk or  $NLR < 3.13$  was 33 subjects (39.8%).

The characteristics of the length of stay for COVID-19 patients who were the subjects of the study are shown in **Table 3**.

The results, presented in **Table 3**, shows the length of hospital stay of subjects with a cut-off value of 11 days. The majority of subjects were found in the group with length of stay  $\leq 11$  days with 58 subjects (69.9%). The number subjects with length of stay  $> 11$  days was 25 subjects (30.1%).

**Table 1. The general characteristics of subjects.**

Category	Frequency (n)	Percentage (%)
Gender		
Male	44	53
Female	39	47
Age (year)		
<5	3	3,6
5-11	1	1,2
12-16	2	2,4
17-25	7	8,4
26-35	16	19,3
36-45	12	15,7
46-55	12	14,5
56-65	15	18,1
>65	14	16,9

Note : Age group classified based on Ministry of Health of Indonesia<sup>8</sup>

**Table 2. The neutrophil lymphocyte ratio of subjects.**

Neutrophil Lymphocyte Ratio	Frequency (n)	Percentage (%)
<3,13	33	39,8
$\geq 3,13$	50	60,2
Total	83	100

Note : <3,13 = low risk,  $\geq 3,13$  = high risk<sup>9</sup>

The correlation between the variables was analyzed through a bivariate analysis. The variables in this study are the NLR value and length of stay in COVID-19 patients at Dr. Zainoel Abidin General Public Hospital Banda Aceh. The correlation between variables in this study was tested using the Spearman Rank test with the SPSS application program. The results of the bivariate test in this study are shown in **Table 4**.

**Table 4** shows the results of Spearman Rank test of the variables. Testing the correlation between NRL and length of stay, conducted with the help of SPSS software, yielded a significance value/p-value of 0.003. A significance value < 0.05 indicates that the null hypothesis is rejected, or the results demonstrated that the NRL value was correlated with length of stay in COVID-19 patients at Dr. Zainoel Abidin General Public Hospital. The correlation coefficient value of the test was 0.319 that indicates a weak correlation between the variables.

## DISCUSSION

The data obtained from **Table 1** indicates that the majority of subjects are male, with 44 subjects (53%), while there are 39 female subjects (47%). The current results reinforce the conclusions obtained in the research undertaken by Daud et al. (2022) on patients tested for COVID-19 at the Bitung City Health Office in 2020. The research found that 109 subjects (59.6%) out of 183 respondents who tested positive for COVID-19 were male.<sup>10</sup> Research by Seftiya et al. (2021) in North Kalimantan showed that 1808 subjects (57.5%) out of 3146 COVID-19 subjects were male.<sup>11</sup> Study conducted by Maryati et al. (2022) in Central Java also showed similar results, with 280 (55.9%) of COVID-19 patients are male.<sup>12</sup>

The higher prevalence of COVID-19 among males is influenced by the X chromosome, which is more dominant in females (XX) than in males (XY). The effect of the X chromosome on the prevalence of infections like COVID-19 is due to the association between the X chromosome and various genes that regulate innate and adaptive immunity.<sup>11</sup> The X chromosome has various effectors necessary for the transcription and translation processes

**Table 3. The length of stay of subjects.**

Length of Stay	Frequency (n)	Percentage (%)
≤11 day	58	69,9
>11 day	25	30,1
Total	83	100

**Table 4. The correlation between NRL and length of stay.**

NRL	Length of Stay				Total		p	r
	≤11 day		>11 day		n	%		
	n	%	n	%				
<3,13	29	34,9	4	4,8	33	39,8	0,003	0,319
≥3,13	29	34,9	21	25,3	50	60,2		
Total	58	69,9	25	30,1	83	100		

Note : NRL = neutrophil lymphocyte ratio, n = number, % = percentage, p-value = significancy value, r = correlation coefficient.

to activate cytokine receptors that play a role in immunity system. Another factor influencing the prevalence of COVID-19 by sex is the difference in sex hormones between men and women. The hormone estrogen in women has the ability to act as an immune activator. The hormone estrogen can trigger the immune system by increasing the regulation of the pro-inflammatory cytokine, tumor necrosis factor/TNF- $\alpha$ . The hormone testosterone, which is dominant in men, can suppress the immunity by increasing the anti-inflammatory regulation by interleukin/IL-10.<sup>13</sup> The difference in prevalence between men and women is supported by WHO data from 2017, which states that the percentage of adult men who are active smokers in Indonesia is high, at 64.9%. Cigarettes contained nicotine that could be the activator of angiotensin-converting enzyme-2 or ACE-2 receptors. The fact indicates that smokers could have more ACE-2 receptors. ACE-2 receptors have been studied in recent studies that found it has major role in the pathogenesis of COVID-19 as the binding site for the SARS-CoV-2 virus.<sup>14</sup> The abundance of ACE-2 receptors in active smokers and men can be linked to the higher rate of COVID-19 positive in the groups.

Table 1 shows the difference in the number between the male group (44 subjects) and the female group (39

subjects), with a small difference. This result is consistent with study by Daud et al. (2022) that found no correlation between gender and the incidence of COVID-19.<sup>10</sup> Study by Putri et al. (2021) also showed similar results, indicating an equal risk of COVID-19 infection for both men and women.<sup>15</sup> Biological states between men and women could be the differences in COVID-19 clinical outcome, but these differences are not a risk factor for COVID-19 incidence. Study by Jin et al. (2020) found that clinical deterioration and mortality rates were higher in the male group. This study confirms that there is no significant difference in incidence between the male and female groups.<sup>16</sup>

The most frequent characteristic of the research subjects was found in the 26–35 age group, with 16 subjects (19.3%), followed by the 56–65 age group with 15 subjects (18.1%). The results demonstrated a similar trend with study by Elviani et al. (2021) in Palembang on patients admitted to the COVID-19 isolation room at Dr. M. Hoesin Hospital Palembang in 2020. The study by Elviani et al. found that the 26–35 age group was the largest, with 74 subjects (23.9%), and the 6–11 age group was the smallest, with 1 subject (0.3%) out of 310 COVID-19 positive confirmed patients.<sup>17</sup> Study by Nurhasnanti et al. (2022) at Sultan Agung Islamic Hospital in Semarang showed that 23 (46%) subjects

were 18–39 age.<sup>18</sup> Study conducted by Halil et al. (2022) at Dr. Chasan Boesoirie Hospital in Ternate showed similar results, with the 30–49 age group were 195 (60%) of total subjects.<sup>19</sup>

Elviani et al. (2021) obtained that the 26–35 age group had the highest number of confirmed COVID-19 cases. The 26–35 age group is considered as productive age. The productive age group has higher mobility and outdoor activity level than other age groups. This results in a higher frequency of social interaction within the productive age group.<sup>17</sup> Transmission of COVID-19 infection is relatively more common in groups with high mobility. Higher mobility and asymptomatic rates in the productive and young age groups result in more transmission and infection rates of infection in these age groups.<sup>12</sup>

The results in Table 1 showed a small difference in the number between the young and old age groups in the study subjects. This is evidenced by the second highest age-based prevalence being the 56–65 age group with 15 subjects (18.1%). The result is consistent with Elviani et al. (2021) that neither found major correlation of age nor the incidence of COVID-19. The elderly age group is also more vulnerable to infection than younger age groups.<sup>17</sup> The vulnerability is consistent with the decline in immune system because of the aging process. The high comorbidity and medication use in the elderly could affect the immune system due to the decline in organ function. Another factor influencing vulnerability in the elderly is the expression of the ACE-2 receptor, which is found to be higher in those over 50 years old due to decreased immunity, organ function decline, and comorbidities. The pathophysiological factor indicated that the elderly age group need more attentions in the pandemic cases.<sup>15</sup>

The most subjects was found in the high risk group or  $NRL \geq 3.13$ , with 50 subjects (60.2%). The results has similar pattern with the result by Khairunnisa et al. (2022) at Dr. H. Abdul Moeloek Regional General Hospital in Bandar Lampung. The study by Khairunnisa et al. (2022) found that 48 out of 74 COVID-19 cases (64.9%) had an NRL value  $> 3.13$ .<sup>20</sup> Study by Kelana et al. (2021) at Wava

Husada Hospital in Malang showed that 18 out of 21 COVID-19 patients (85.7%) had  $NRL \geq 3.13$ .<sup>21</sup> Study by Hasanah et al. (2022) at Nganjuk Regional General Hospital showed consistent results, with 74% of 38 COVID-19 patients had  $NRL > 3.13$ .<sup>22</sup>

Several hematological test alterations have been identified in association with the pandemic infection. The altered hematological parameters include white blood cell count values, particularly neutrophilia and lymphocytopenia. These changes in both parameters are characterized by an increase in the neutrophil lymphocyte ratio (NLR). These changes in both hematological parameters can be found earlier when patients receive initial treatment at healthcare facilities.<sup>23</sup> Increased production of cytokines such as granulocyte colony-stimulating factor (G-CSF), granulocyte-monocyte colony-stimulating factor (GM-CSF), and monocyte colony-stimulating factor (M-CSF) triggers granulopoiesis, resulting in the production of new granulocytes that will be recruited to the site of inflammation, primarily neutrophils. Neutrophil infiltration in pulmonary capillaries accompanied by increased expression of neutrophil-related chemokines is found in COVID-19 cases.<sup>24</sup> This leads to neutrophilia, which is also triggered by the immune system due to early viral evasion in cases of COVID-19 infection. This neutrophilia manifests as dysregulation of the inflammatory process and cytokine storm, which can cause hyperinflammation and tissue damage.<sup>25</sup> Another hematological parameter found in COVID-19 is lymphocytopenia, similar to other viral infections.<sup>26</sup> Differences that can be found in COVID-19 are more selective lymphocytopenia, which occurs in T lymphocytes. Lymphocytopenia in COVID-19 is influenced by the recruitment of lymphocytes to the respiratory tract or the adhesion of lymphocytes to inflamed vascular endothelium. Another event contributing to lymphocytopenia is the increased expression of Fas protein, TNF-related apoptosis-inducing ligand, and caspase 3 that were apoptotic inducer.<sup>27</sup> Changes in hematological values, such as neutrophilia and lymphocytopenia, lead to changes in the NRL value, which

is commonly found to be elevated in COVID-19 cases.<sup>28</sup> The NRL value as an inflammatory marker is not only used in COVID-19. The NRL value has been used in infectious, autoimmune, cardiovascular, and malignant diseases.<sup>29,30</sup> Changes in the balance between the number of neutrophils or lymphocytes, such as an increase in the NRL value, are found in the 4 pathological conditions recently mentioned.<sup>31–34</sup>

The results also showed that 33 subjects (39.8%) had been included in low risk group or  $NRL < 3.13$ . The NRL assessment obtained from complete blood count examination at the time the subjects were initially admitted to the hospital. A study by Fuad et al. (2021) in Banda Aceh also assessed NRL in COVID-19 based on blood tests performed at the initial time of the hospitalization. The study by Fuad et al. found that the NRL value of mild infection were lower than severe and critical infection. The study showed that the median NRL value in the mild group was 2.27 compared to 6.54 in the severe group.<sup>5</sup> Research by Toori et al. (2021) on confirmed positive patients in Pakistan also showed similar findings. The study found that the NRL value was lower in patients without symptoms and with mild disease. This indicates the benefit of using the NRL value as a prognostic marker to differentiate between mild and severe COVID-19 patients in the early stages of treatment.<sup>35</sup>

The most frequent length of stay in the subjects was found in the group with  $\leq 11$  days, with 58 subjects (69.9%). The results has similarities with results of research by Kurnia et al. (2022) at Medan Haji General Hospital on COVID-19 patients from 2020 to 2021. The study by Kurnia et al. (2022) found that 648 subjects (91.1%) out of 711 COVID-19 patients were hospitalized for  $\leq 11$  days.<sup>36</sup> Research by Maharianingsih et al. (2022) at Karangasem Regional General Hospital in Bali showed that 138 subjects (69%) out of 200 COVID-19 patients from April 2020 to April 2021 were hospitalized for 0–7 days.<sup>37</sup> Research by Jamini (2021) at H. Boejasin Regional General Hospital in Palaihari showed consistent results, with 158 (76%) out of 208 COVID-19 patients being hospitalized for  $\leq 14$  days.<sup>38</sup>

The hospitalization period of the

pandemic case varies widely across the world. Study by Rees et al. (2020) found that the median length of stay in China was 14 days compared with outside of China that was 5 days. This difference was estimated occurred by varying admission and discharge criteria used in each country.<sup>39</sup> Study by Arief et al. (2021) at Yarsi Hospital in Jakarta showed that 179 patients (81%) of the COVID-19 cases studied were asymptomatic or had mild symptoms.<sup>40</sup> Study by Alahmari et al. (2022) on factors influencing length of hospital stay in Saudi Arabia found that patients with severe and critical symptoms have more longer length of stay period than asymptomatic or mild symptoms, which were the dominant cases of COVID-19.<sup>41</sup> The vaccination program is also expected to impact the COVID-19 pandemic. Full dose vaccination coverage in Indonesia reached 55.61% and partial dose coverage reached 80.83% in January 2022. Indonesia was the fourth country in the world with the largest vaccination coverage.<sup>42</sup> Study by Havers et al. (2022) found that the median length of stay for the vaccinated group was shorter (4.3 days) than for the unvaccinated group (4.6 days).<sup>43</sup> The shorter hospital stay in the vaccinated group can be attributed to the higher SARS-CoV-2 antibody titer compared to the unvaccinated group.

The results of the Spearman Rank test in this study indicates a correlation between NRL and length of stay in the subjects, with a significance value/p-value <0.05. The results are consistent with research conducted by Baihaqi et al. (2021) at Serui Regional General Hospital in Papua on 96 COVID-19 patients from September 2020 to August 2021. The study by Baihaqi et al. (2021) found a significant correlation between NRL and length of stay, with a significance value (p-value) of 0.01.<sup>44</sup> Study by Isbaniah et al. (2021) at Persahabatan General Hospital in Jakarta showed a correlation between NRL values and length of stay in studied subjects from March to July 2020, with a p-value of 0.005 and a correlation coefficient (r) value of 0.269.<sup>45</sup> Ertekin et al. (2021) studied on 500 subjects in Turkey showed consistent results, indicating a correlation between NRL values and length of hospital stay with a p-value of 0.000 and r of 0.632.<sup>46</sup>

Neutrophils has major contribution

in the pathogenesis and complication of COVID-19. Neutrophil extracellular traps (NETs) that secreted by neutrophil was pathogens destroyer. NET generation called NETosis was the processes associated with cell death. NETosis can also worsen the inflammatory response and thrombosis due to infection. The infection process by COVID-19 could lowering B and T cell in the serum, especially in severe and critical case The effect on lymphocytes disrupts adaptive immunity function in fighting infection. Neutrophilia and lymphocytopenia are immunological dysregulations that can lead to cytokine storms and other infectious complications such as acute respiratory distress syndrome (ARDS) and multi-organ failure (MOF).<sup>47-49</sup>

The NRL examination provides an overview of the neutrophil and lymphocyte balance in COVID-19 patients, especially in initial hospitalization. NRL testing for COVID-19 cases can be done easily and quickly because it is included in the routinely performed hematological parameters. The ability of the NRL value indicates the test capability in assessing systemic inflammation occurring in COVID-19, so that could be one of the screening tests for COVID-19.<sup>47</sup> Study by Zhang et al. (2020) found that the NRL value was also positively correlated with the extent of lung lesions in COVID-19, as measured by the computed tomography severity score. This correlation provides positive benefits for healthcare professionals in assessing clinical conditions, especially in facilities where radiology services are limited.<sup>50</sup> Study by Ai Ping et al. (2020) found that an increase in neutrophils is directly proportional to the worsening of the disease in COVID-19, indicating neutrophil as a useful independent biomarker for assessing clinical outcomes.<sup>51</sup> The various positive uses of NRL in describing clinical conditions play an important role for healthcare professionals when treating patients, enabling them to provide appropriate therapy, adjust therapeutic needs, and monitor the onset of complications in COVID-19 patients.<sup>52</sup> This facts was supported by the study by Jin Hu et al. (2020). The study found that the length of hospital stay for COVID-19

patients was positively correlated with an increase in NRL values.<sup>53</sup> Research by Kulkarni et al. (2020) concluded that NRL assessment in the early phase of COVID-19 therapy is an easily performed, economical, and relatively available marker in almost all healthcare facilities.<sup>47</sup>

## CONCLUSION

In Conclusion, the most common NLR in COVID-19 positive confirmed subjects at Dr. Zainoel Abidin General Public Hospital Banda Aceh was  $\geq 3.13$ . The majority of hospitalized patients with confirmed COVID-19 in the hospital had a length of stay of  $\leq 11$  days. There was a significant correlation between the NLR and the length of hospital stay in COVID-19 patients ( $p = 0.003$ ). The NLR can be used as a predictor of length of stay in COVID-19 patients.

## DISCLOSURES

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### Conflict of Interest

There are no declared conflicts of interest by the authors in relation to the materials or methodologies employed in this research.

### Ethical Approval

Prior to data collection, the authors secured institutional approval from Dr. Zainoel Abidin General Hospital to conduct the research.

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