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NLR and ALC Levels are Key Biomarkers for Distinguishing COVID-19 Cases

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Abstract. This study examines the diagnostic disparities of Neutrophil Lymphocyte Ratio (NLR) and Absolute Lymphocyte Count (ALC) between COVID-19 positive and negative patients. Using a laboratory experimental design with quantitative methods, 54 samples from each group were analyzed. Statistical analysis revealed significant differences in NLR ($p = 0.001$) and ALC ($p = 0.038$) values, with average NLR levels of 8.40 in COVID-19 positive patients and 4.87 in negatives, and average ALC values of 1.44 in positives and 1.80 in negatives. These findings highlight the diagnostic utility of NLR and ALC in distinguishing COVID-19 cases, emphasizing the importance of immune response biomarkers in clinical assessment and management strategies.

Highlights:

1. Significant differences in NLR and ALC values between COVID-19 positive and negative patients.
2. Higher average NLR levels in COVID-19 positive patients.
3. Lower average ALC values in COVID-19 positive patients.

Keywords: SARS-Cov-2, COVID-19, Neutrophil Lymphocyte Ratio, Absolute Lymphocyte Count, Immune Response

Introduction

The SARS-C o V-2 virus is the cause of Coronavirus Disease 2019 (Covid -19) which shocked the world in December 2019. Covid -19 is a new type of disease that has never been previously identified in humans, This new virus was first detected in a laboratory in Wuhan China which spreads through saliva droplets and nasal fluids , This virus causes acute respiratory problems such as fever, cough and shortness of breath. In severe cases it can cause pneumonia, acute respiratory syndrome and even kidney failure. The SARS-COV-2 virus belongs to the group of viruses of the Order Nidovirales with non-segmented positive-strand RNA and is also classified as zoonotic, namely a virus that can be transmitted between animals and humans [1]. Coronavirus is a virus with single chain RNA with a lipid envelope, positive sense with a diameter of 60-140 nm, which can be said to be almost 100 times smaller than the average cell in the human body. Coronavirus is included in the subfamily Ortho Coronaviridae, based on the genus, it has four types namely alpha coronavirus, beta coronavirus, gamma coronavirus and delta coronavirus [2].

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On January 30 2020, WHO declared Covid -19 a public health emergency that was of global concern and declared Covid -19 a pandemic on March 11 2020. The first case in Indonesia occurred on March 2 2020, there were 2 confirmed cases of Covid -19 patients in Jakarta. complained of cough, fever and shortness of breath [1]. During March to August 2020, 67.79% of cases recovered and 4.83% died, most of the patients were male 50.52% and adults in the age range 31 – 45 years 29.73%. Initially infected with the virus there are no symptoms in the body or are asymptomatic, then mild symptoms will appear such as coughing, fatigue, anorexia, shortness of breath and myalgia. In this condition the oxygen saturation is > 95%, with moderate symptoms there are clinical signs of pneumonia in patients > 5 years old. rapid breathing > 30 times per minute, severe symptoms followed by severe pneumonia with respiratory rate > 30 times per minute and saturation

Oxygen < 93% severe respiratory distress and in the critical phase acute respiratory distress syndrome (ARDS), sepsis and septic shock are found which can be interpreted as requiring life support equipment such as mechanical ventilation [4].

The virus passes through the mucous membrane through the nose and larynx to enter the lungs in the respiratory tract , the virus expresses Angiotensin Converting Enzyme 2 (ACE2) to attack target organs, such as the lungs, heart, renal system and gastrointestinal tract , Covid-19 incubates for 3 Up to 14 days initially the body does not feel the symptoms of a virus attack until the virus has spread through the bloodstream and attacks organs that express ACE2. The body begins to feel mild symptoms within 7 days of the initial attack, and if the condition continues to worsen the patient will feel short of breath until respiratory failure. [5]

SARS-C o V-2 virus infection can produce an excessive immune reaction in humans. The overall reaction is called a cytokine storm, which is an excessive inflammatory reaction in which there is rapid production of large amounts of cytokines in response to infection. When inflammation occurs, the body will respond physiologically in the form of a decrease in the number of lymphocytes and an increase in the number of neutrophils in the body. Therefore, the development of biomarkers to present inflammatory and immune status is very useful for the prognosis of Covid -19 patients [7]. Neutrophil Lymphocyte Ratio (NLR) is a blood test as a marker for inflammation, calculating the number of NLR from the differential white blood cell component, namely dividing the

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number of neutrophils by the number of lymphocytes , in the body the number of neutrophils is very large and functions as a defense against viruses through internal release of viruses, a killing mechanism , release of cytokines, degranulation and neutrophil extracellular tras (NETs), when inflammation occurs cellular immunity will reduce levels of T helper lymphocytes (CD4)

and increasing cytotoxic lymphocytes (CD8) causes the ratio of neutrophils to lymphocytes to increase [8]. Absolute Lymphocyte Count (ALC) is a blood test to determine the number of lymphocytes in the blood. When an infection occurs in the body such as a virus, cancer or autoimmunity, it can cause changes in the value of lymphocytes . In the case of Covid -19 positive patients, there is often a decrease in lymphocytes where the lymphocytes express the ACE2 receptor. and binds to the SARS-CoV-2 virus in cells and causes lysis and a cytokine storm [5].

Polymerase Chain Reaction (PCR), a technology that can multiply DNA fragments and has quite high sensitivity because it only requires a small DNA sample, was first discovered by Kary Banks Mullis 32 years ago. Theoretically using the formula Y = (2n – 2n) DNA strands use high temperatures, second annealing reduces the temperature to facilitate the attachment of DNA that is complementary to the primer, then polymerization where the single strand of DNA is read by DNA polymerase by adding DNA bases so that DNA fragments can be multiplied exponentially [7].

According to research by Danis Pertiwi (2022) entitled the relationship between Neutrophil Lymphocyte Ratio (NLR) with the mortality of Covid -19 patients shows that the severity of Covid -19 is influenced by the body's innate inflammatory response and is related to cytokine storms which can trigger death , in severe degrees the number of lymphocytes decreases progressively and conversely the number of neutrophils increases [10]. According to research by Khamidun Nisak (2022) entitled Correlation of Absolute Lymphocyte Count (ALC) values with mortality in Covid-19 patients in the early stage, ALC and NLR values are normal. Entering the peak of the incubation period (day 14) the ALC value decreases and the NLR will increase. However, after treatment, the NLR value decreased and showed good indications [11]. Therefore, it is necessary to conduct research on the differences in the diagnostic values of NLR and ALC in Covid-19 positive patients and Covid-19 negative patients.

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Method

This research was carried out after getting p a t letter information get away test e type from Bangil D eng a n Regional Hospital number 445.1/14/424.072.01/2023 . This research uses a laboratory experimental research design, with quantitative methods. The research design used aims to determine the difference in diagnostic values of NLR and ALC in positive and negative Covid-19 patients at B Angil Regional Hospital . Study This use taking sa m p e l simple random sampling And held on March 2023 . S a mpel on study This that is 54 patients were confirmed positive for Covid -19 and 54 patients were negative for Covid -19. Taking samp e l And inspection done in Bangil Regional Hospital uses it a Haemotology Analyzer tool (Cell Dyn Ruby) with method Multi-Angle Polarized Scatter Separation (MAPSS)

Reslt and Discussion

The percentage of CT Value data from 54 positive Covid-19 patients was found to be 16% (9 patients) at CT Value 11 - 20, 80% (43 patients) at CT Value 21-30 and 4% (2 patients) at CT Value 31- 40, whereas in patients negative for Covid-19 there were no patients with CT values as in Table 1.

Table 1. Frequency distribution of positive Covid-19 patients and negative Covid - 19 patients at Bangil Regional Hospital based on n

CT Value value.

		Positive Patient		Negative Patient	
	gree of CT Value	Frequency	Percentage (%)	Frequency	Percentage (%)
1	11-20	9	16	0	0
2	21-30	43	80	0	0
3	31-40	2	4	0	0
	Total	54	100	0	0

Source: Primary Data, 2023

In the results of the Mann-Whitney Statistical Test, the NLR value for Covid-19 positive and negative patients showed a mean value of 8.40 for Covid-19 positive patients and a mean of 4.87 for Covid-19 negative patients and a significant value of 0.001 as in Table 2.

Table 2 . Statistical Test of Differences in Neutrophil Lymphocyte Ratio (NLR) Results in Positive Patients and Negative Patients

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Covid-19 at Bangil Regional Hospital

		Group	
		Covid Positive Patient	Covid Negative Patient
NLR Results	Mean	8 , 40	4 , 87
	elementary school	6 , 73	3 , 72
	Minimal	1 , 45	0 , 62
	Max	32 , 78	15 , 50
	Amount	453 , 96	263 , 44
	Mann-Whitney	Asymp Sign. (2-tailed) : 0 , 001	

In the results of the Mann-Whitney Statistical Test, the ALC value for Covid-19 positive and negative patients showed a mean value of 1.44 for Covid-19 positive patients and a mean of 1.80 for Covid-19 negative patients and a significant value of 0.038 as in Table 3.

Table 3 . Statistical Test of Differences in Absolute Lymphocyte Count (ALC) in Positive Patients and Negative Patients

Covid-19 at Bangil Regional Hospital

		Group	
		Covid Positive Patient	Covid Negative Patient
ALC results	Mean	1 , 44	1 , 80
	elementary school	0 , 82	0 , 92
	Minimal	0 , 31	0 , 32
	Max	3 , 77	4 , 53

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	Amount	77 , 91	97 , 21
	Mann-Whitney		Asymp Sign. (2-tailed) : 0 , 038

The results of the Kolmogrov Smirnov normality test obtained a significance value of 0.000, where the sig value <0.05 can be interpreted as not normally distributed data. So a non-parametric test was carried out to determine the difference in NLR and ALC values in Covid-19 positive patients and Covid-19 negative patients using the Mann Whitney statistical test. In the Mann Whitney test, a significant value for NLR was obtained, namely 0.001, where the sig value was <0.05, so it could be concluded that there was a difference in the NLR value for Covid-19 positive and Covid-19 negative patients. Meanwhile, the ALC value obtained a significance value of 0.038, which means the sig value is <0.05, so it can be concluded that there is a difference in the ALC value in positive Covid-19 and negative Covid-19 patients.

Based on research data, the NLR was found to be 8.40 (patients positive for Covid-19) and 4.87 (patients negative for Covid-19) while the ALC value was 1.44 (patients positive for Covid-19) and 1.80 (patients negative for Covid-19) this is in line with Danis Pertiwi's research in 2022 which stated that the NLR value in Covid-19 positive patients had increased because the SARS-CoV-2 virus which binds ACE2 was responded to by the body through innate immunity which was then expressed by increasing neutrophils and macrophages to killing pathogens, viruses and destroyed infected cells will cause tissue damage which triggers the release of cytokines and causes widespread inflammation resulting in increased NLR values, high NLR values indicate an imbalance in the inflammatory response, systemic inflammation triggered by the SARS-CoV-2 virus will cause apoptosis lymphocytes and suppress CD4 and CD8 cellular immunity which makes ALC values decrease.

In the Khamidun Nisak study in 2022, in the early stage, the ALC and NLR values were normal. Entering the peak of the incubation period (day 14) the ALC value decreases and the NLR will increase. However, after treatment, the NLR value decreased and showed good indications.

Neutrophils are an important component in leukocytes that actively go to the immune organ system. If there is a virus, neutrophils will produce large amounts of Reactive Oxygen Species (ROS) to induce damage to the cell's DNA. causes the virus to leave the cell, then Antibody Dependent Cell Mediated Cell (AICDCC) will attack the virus and cause humoral immunity. A high NLR value can determine the level of severity and predict a poor prognosis so that it can identify the risk of Covid -19 patients to receive appropriate medication to reduce the number of deaths in hospital. The ALC value can describe the pathological condition of Covid -19 patients as a marker of the severity of the infection . An increase in neutrophils and a decrease in lymphocytes reflects an

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imbalance between the non-specific immune response (neutrophils) and the adaptive immune response (lymphocytes), in severe cases a high NLR value indicates severe dysregulation of the immune system and an excessive non-specific immune response . The process of decreasing lymphocytes where the T cell response is disrupted during acute SARS-CoV-2 infection occurs when macrophages are activated, T cells and B cells will respond by producing pro-inflammatory cytokines and chemokines, and this will encourage depletion and weakening of T cells which are influenced by mature dendritic cells. which migrate to lymphoid organs to activate T cells which can cause lymphocytopenia .

Conclusion

Based on the research results, it can be concluded that there is a difference in the NLR value ($p = 0.001$) and ALC value ($p = 0.038$) in Covid-19 positive and Covid-19 negative patients. The average NLR level in Covid-19 positive patients was 8.40 and in Covid-19 negative patients was 4.87. The average ALC value in Covid-19 positive patients was 1.44 and in Covid-19 negative patients was 1.80.

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