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Diagnostic Test of Neutrophil Lymphocyte Ratio in Confirmed Covid-19 Positive Patient at RSUD Wonosari

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ABSTRACT

Coronavirus Disease 2019 (Covid-19) is an outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The neutrophil lymphocyte ratio (NLR) biomarker is used as a parameter of inflammatory reactions which is also frequently evaluated in Covid-19. In Covid-19 patients, there is often an increase in NLR through the mechanism of lymphocyte exhaustion. This research to identify the performance of NLR in a diagnostic test for confirmed Covid-19 patients at RSUD Wonosari. This study is analytical observational through a cross-sectional approach. Sampling used a consecutive sampling technique. The target population was suspected Covid-19 patients. Data were analyzed by univariate, ROC curve, and diagnostic tests. A total of 75 suspected Covid-19 patients were found 33 male patients (44%), 42 female patients (66%), and the most aged> 55 years were 52 patients (69.3%). RT-PCR results found 57 patients were positive for Covid-19 (76%) and 18 patients were negative for Covid-19 (24%). ROC curve obtained AUC value 0.785 (p<0.05; 95% CI 0.662-0.907) with cut-off point NLR≥ 2.33, sensitivity 91.2%, specificity 50%, NPV 64.3%, PPV 85.2%, LR+ 1.81, and LR- 0.17. NLR can be an initial diagnostic tool for Covid-19.

KEYWORDS:
Covid-19, Diagnostic test, NLR, RT-PCR

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INTRODUCTION

Pandemic Coronavirus Disease 2019 (Covid-19) is an outbreak of disease caused by the Coronavirus or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Suspected viruses originate from traditional markets in the Chinese city of Wuhan and spread with fast Because can be transmitted between humans (human-to-human transmission). As this February, 2026, already reach figure of 779,073,803 cases confirmed Covid-19 positives worldwide by WHO and figures death as many as 7,109,103 cases. In Indonesia itself Already confirmed case confirmed 6.663.182 cases positive for Covid-19 and figures death as many as 6.663.182 cases.[1]

The diagnostic method recommended by WHO to diagnose Covid-19 is the molecular detection method/NAAT (Nucleic Acid Amplification Test) such as RT-PCR examination. RT-PCR examination has the advantage of having high sensitivity and specificity and being able to detect antigens at low concentrations. However, this examination takes a long time, is quite expensive, must have a special applicator, and variations in samples can change the

results of the examination. In this case, it is necessary to consider other examinations such as thoracic tomography or calculation of the profile of the neutrophil lymphocyte ratio (NLR).[2]

Checking the NLR value has the advantage that it is easy to obtain by inspection alone Complete Blood Count (CBC), is affordable, simple, more efficient, and inexpensive. However, it has drawbacks where NLR values can also increase in other circumstances such as sepsis, long-term use of corticosteroids, acute myocardial infarction, emotional stress, and pregnancy.[2] In research on 2021 with performance NLR cut-off point > 2.33 have a marked sensitivity 75%, specificity 60%, and AUC within level moderate accuracy 0.723 in diagnosing Covid-19.[3] The study evaluated the effectiveness of the Neutrophil-to-Lymphocyte Ratio (NLR) and Lymphocyte-to-Monocyte Ratio (LMR) as diagnostic parameters for COVID-19 diagnosis. The analysis revealed an optimal NLR cut-off value of ≤2.49, yielding a specificity of 80%, sensitivity of 47.6%, positive predictive value (PPV) of 69%, and negative predictive value (NPV) of 62.1%, with an Area Under the Curve (AUC) of 0.638[13]. Furthermore, this study aims to fill the existing research gap regarding the diagnostic accuracy of NLR in the Indonesian clinical setting, specifically within the Yogyakarta region, where localized data remains limited. The primary objective is to validate whether NLR can serve as a reliable and accessible alternative for early COVID-19 diagnostic tools in secondary healthcare facilities, such as RSUD Wonosari, by comparing its performance directly against the gold standard RT-PCR results.

METHODS

Study Design

This observational study approach cross-sectional design.

Setting and Respondents

Retrieval process sample use technique of consecutive sampling conducted from September 2021 to October year 2022. The accumulated results sample will be tested using analysis univariate and diagnostic tests with source sample form of secondary data from record status medical patients confirmed Covid-19 being treated at Wonosari Hospital. Population is patient suspected of Covid-19 at the Wonosari Hospital. The inclusion criteria in this study are patient suspected of Covid-19 aged over 18 years, have results of RT-PCR Covid-19 examination, and have results of complete blood count (CBC) that includes mark neutrophils and lymphocytes. Research exclusion criteria are patients with pregnancy and patients with immunodeficiency. While various clinical conditions can influence the Neutrophil-to-Lymphocyte Ratio (NLR), this study specifically prioritized pregnancy and immunodeficiency as primary exclusion criteria due to their profound and consistent impact on systemic leukocyte distribution, which would

significantly skew the baseline data. The sample size in the study was 77 people with 2 samples excluded Because pregnant so the total sample used as many as 75 people. The predictor variables in this study were the cut-off point NLR ≥ 2.33 referring to the research by Özsari S, sensitivity, and specificity of the NLR of Covid-19 patients, while the outcome variable was the RT-PCR status of Covid-19 patients. Data was analyzed in a manner univariate, ROC curve, and diagnostic tests with the application IBM Statistical Package for the Social Sciences (SPSS) Statistics 25. This research has received ethical approval from the Research Ethics Committee of RSUD Wonosari.

The Variables, Instruments, and Measurement

The instrument used in this study is a structured form containing age, gender, symptom clinical, comorbid, outcome CBC examination, RT-PCR Covid-19 status along with date inspection. Complete Blood Count (CBC) was performed using an automated hematology analyzer to determine leukocyte, neutrophil, and lymphocyte levels. The molecular diagnosis was confirmed via RT-PCR. Viral RNA was extracted from nasopharyngeal and oropharyngeal swabs, with the presence of SARS-CoV-2 determined by the amplification of specific target genes (e.g., ORF1ab, N, and E genes) according to the manufacturer's protocol. The sample is secondary data on patient medical records at Wonosari Hospital.

Data Analysis

The diagnostic test was analyzed using a Receiver Operating Characteristic (ROC) curve which is a yield curve between sensitivity and specificity at various cut points. From this analysis the researcher will get the value of the cut-off point which is the normal, abnormal, or cut-off point for positive or negative test results for a disease with the optimal cut-off point. This analysis is used to assess the ability of a diagnostic test whose measurement results are continuous scale to detect the presence of a disease.

In addition, the value of Area Under the Curve (AUC) can be obtained where AUC is the entire area under the curve formed from all sensitivity and 1-specificity coordinates. The application of the diagnostic test starts from inputting the NLR value of suspected Covid-19 patients and RT-PCR status which then determines the optimal cut-off value by looking at the correct value classified is the highest. After obtaining the optimal cut-off value, sensitivity, specificity, LR+, and LR-. Then proceed with an analysis using a 2x2 table to calculate the performance.

RESULT AND DISCUSSION

Of the 223 patient medical records taken at Wonosari Hospital, a total of 75 research subjects met the inclusion and exclusion criteria. The detailed sample profile is described in Table 1.

Table 1. Characteristics of Respondent

Characteristic	Result
Age, years	
19-35	10 (13.4 %)
36-55	13 (17.3%)
>55	52 (69.3%)
Gender	
Male	33 (44%)
Woman	42 (56%)
Comorbid	
Hypertension	19 (25.3%)
Cardiovascular-Hypertension	21 (28%)
Cardiovascular-Hypertension-Dyslipidemia	1 (1.3%)
Cardiovascular-Hypertension-COPD	1 (1.3%)
Cardiovascular-Hypertension-CKD	1 (1.3%)
Cardiovascular-Hypertension-DM	1 (1.3%)
Dyslipidemia	3 (4%)
Dyslipidemia-Hypertension	3 (4%)
Pneumonia	2 (2.7%)
CKD	1 (1.3%)
CKD-Hypertension	4 (5.3%)
DM	5 (6.7%)
DM-Hypertension	3 (4%)
DM-Hypertension-CKD	1 (1.3%)
Without Comorbidities	9 (12%)
Symptom	
Acute Respiratory Infections	19 (25.33%)
Non-Acute Respiratory Infections	1 (1.33%)
Mix	51 (68%)
No Symptom	4 (5.34%)
RT-PCR	
Positive	57 (76%)
Negative	18 (24%)

As shown in Table 2, the normality test using the Kolmogorov-Smirnov method indicated that the data were not normally distributed ($p < 0.05$). Consequently, the leukocyte, the absolute number of neutrophils, lymphocyte absolute numbers, neutrophil-lymphocytes ratio and platelet counts are presented as median values with their respective minimum and maximum ranges using IBM Statistical Package for the Social Sciences (SPSS) Statistics 25. Based on the analysis of 75 research subjects using IBM SPSS, the ROC curve presented in Figure 1 was generated. From this analysis, it can be concluded that applying the optimal cut-off point of ≥ 6.305, derived from the intersection curve in Figure 2, yields an AUC of 0.785. This indicates a 78.5% probability that the NLR test will correctly distinguish between a randomly selected positive patient and a negative patient. This favorable diagnostic performance is further reflected by a specificity of 72.22% and a sensitivity of 70.17%.

Table 2. Leukocyte and Platelet Profiles.

Parameter (10^3/uL)	Median	Minimum	Maximum	Recommendation
Leukocytes	7,9	2,5	28,89	4,40-11,50
Absolute Neutrophil Count	6,63	1,83	27,44	2,30-8,60
Absolute Lymphocyte Count	0,98	0,17	5,31	1,62-5,37
Platelet Count	173	23	665	150-450
Neutrophil-Lymphocyte Ratio	7,08	0,39	32,30	0,78-3,53

In Table 3, the study compares the optimal NLR cut-off point (≥ 6.305) with the hypothesized NLR cut-off point (≥ 2.33) among samples at RSUD Wonosari. The results demonstrate that a cut-off point of ≥ 2.33 yields a sensitivity of 91.2%, indicating its ability to correctly identify 91.2% of true-positive COVID-19 cases. However, the specificity at this threshold was only 50%, reflecting a limited capacity to accurately identify negative cases among those without the infection. The low specificity of 50% at the ≥ 2.33 threshold presents a significant clinical challenge, as it results in a high rate of false-negative cases. This suggests that half of the patients who do not actually have COVID-19 would be incorrectly identified as positive, leading to unnecessary anxiety and the potential for hospital resource strain.

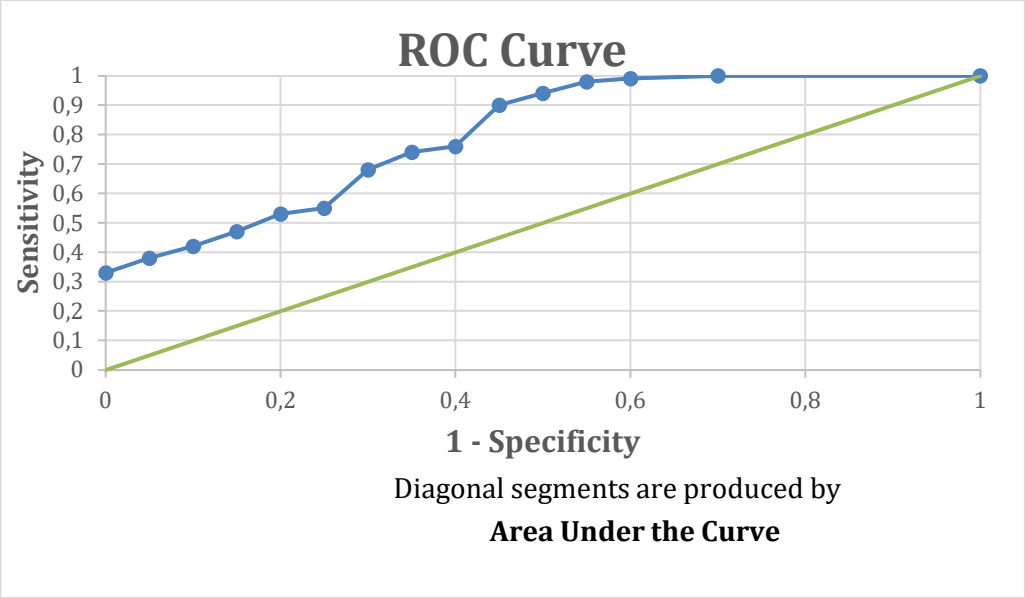

Figure 1. NLR Receiver Operating Characteristic Curve against RT-PCR.

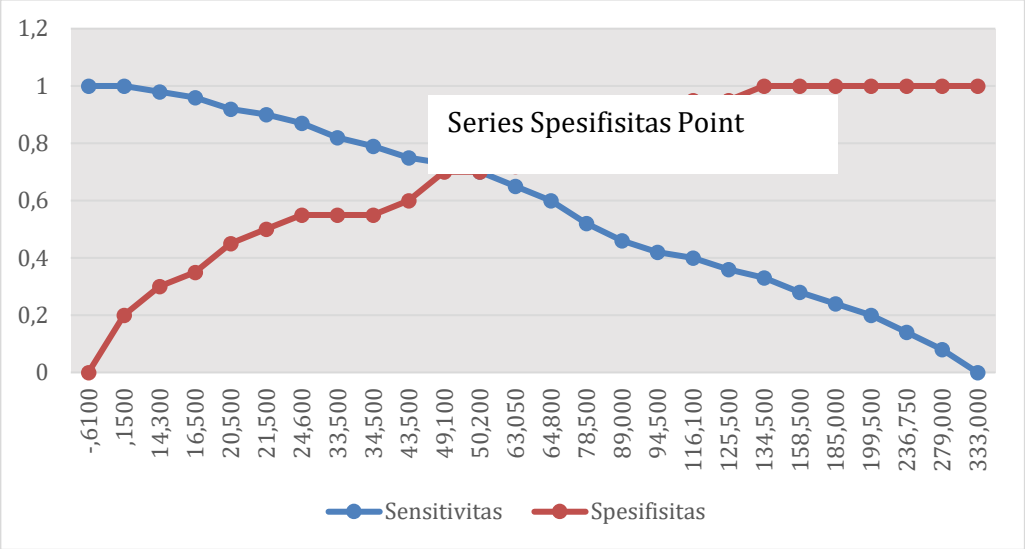

Figure 2. Sensitivity and Specificity Intersection Curves of NLR to RT-PCR in Covid-19 Patients.

Table 3. Cut-off Point Diagnostic Test NLR ≥ 2.33 and RT-PCR Covid-19.

No		RT-PCR		Total
		Positive	Negative	
NLR	≥ 2.33	52	9	61
	< 2.33	5	9	14
Total		57	18	75

Sensitivity : 52/ (52+5) X100% = 91.2%
Specificity : 9/ (9+9) X100% = 50%
PPV : 52/ (52+9) X100% = 85.2%
NPV : 9/ (9+5) X100% = 64.3%
LR+ : 91.2/ (100-50) = 1.81
LR- : (100-91.2)/ 50 = 0.17

According to Ministry of Health of the Republic of Indonesia, 2020. Guidelines for the Prevention and Control of Coronavirus Disease (COVID-19) can use a hematological examination, namely an absolute lymphocyte count with a value of <1500/µL, neutrophils >2500 µL, and an examination neutrophil lymphocyte ratio > 3.13. In addition, examinations such as platelet counts can also be used as a reference. In addition, examinations such as platelet counts can also be used as a reference. Other studies also show that NLR values ≥ 5.8 increase the likelihood of positive RT-PCR results in examining patients with suspected Covid-19[4]

According to Gusti et al., 2021, the absolute number of neutrophils in Covid-19 patients shows a dominance of the normal range of 73.7%, so it is in line with this study.[5] The absolute number of neutrophils was also found to be within the normal range of 3.37 103/uL in a study of 1009 Covid-19 patients in Wuhan, China.[6] The elevation of absolute neutrophil counts in COVID-19 patients is contingent upon the progression of the infection. This can occur when the body's defense by neutrophils causes emergency granulopoiesis. Emergency granulopoiesis is a form of blood formation response which will rapidly increase neutrophil production due to infection which directly increases peripheral blood neutrophils.[7]

The absolute number of lymphocytes in this study has decreased numbers or lymphopenia. These results are in line with the study by Guan et al., 2020, where the results of a complete blood count in most patients showed lymphopenia (83.2%).[8] There are several factors that can explain the relationship between Covid-19 and the incidence of lymphopenia. Viruses can lyse lymphocytes directly, a cytokine storm that causes lymphocyte apoptosis and atrophy of lymphoid organs, and through the mechanism of lymphocyte exhaustion.[7]

The explanation regarding the results of platelet counts with normal ranges in this study is in line with the study of Hayati et al., 2022 which had the results of 46 samples of normal platelet counts from Covid-19 patients with mild, moderate, or severe symptoms from a total of 50 samples.[9] However, other studies have revealed that thrombocytopenia can occur in Covid-19 because the combination of viral infection and mechanical ventilation causes endothelial damage which triggers platelet activation, aggregation and thrombosis in the lungs which causes increased platelet consumption. Thrombocytopenic events usually occur in critically ill patients who show serious organ damage or physiological decompensation, vascular coagulopathy and disseminated intravascular coagulopathy (DIC).[10]

Based on the result shows an increased median NLR of 7.08 among those infected with COVID-19, in line with other studies. NLR in Covid-19 patients showed an increase of 4 ± 3 and was higher in 16 symptomatic patients with 70.3 ± 13.3.[5] However, it is different in the study by Hayati et al., 2022, the dominance of NLR numbers is in the normal range of 26 people out of a total of 50 samples with a p value <0.05.[9] An increase in NLR values can occur due to the Sars-COV-2 virus causing severe inflammation and multiple organ damage due to a cytokine storm (hyperinflammation). This systemic inflammation causes depletion of T lymphocytes, both T-helper lymphocytes and T-suppressor lymphocytes, which is characterized by lymphopenia on a complete blood count (lymphocyte exhaustion).[7]

The results of this study, are in line with the research of Özsari et al., 2021 with an NLR cut off point performance of > 2.33 having a sensitivity value of 75%, specificity of 60%, and AUC at a moderate accuracy level of 0.723.[3] Furthermore, the diagnostic utility of the NLR was supported by a cut-off point of ≥ 2.23, which demonstrated a sensitivity of 87.1% and a specificity of 49.7%. The corresponding Area Under the Curve (AUC) was 0.722, indicating a moderate level of diagnostic accuracy.[11] However, several other studies had low NLR diagnostic test performance with a weak degree of accuracy in the range of AUC values of 0.6-0.7 which were conducted in Turkey and in Medan. In a study in Turkey the performance of the NLR cut off point ≥ 2.4 had a sensitivity value of 69.01%, a specificity of 65.40%, and an AUC of 0.660 at a weak accuracy level in the total 80 samples used.[12] In the study in Medan, the performance of the NLR cut off point ≤2.49 had a sensitivity value of 47.6%, a specificity of 80%, and an AUC of 0.638 at a weak accuracy level in 87 samples.[13] Another study in Pakistan tried to test the value of NLR compared to RT-PCR as an initial diagnostic tool with a cut-off point ≥ 3.5. In 172 patients, a sensitivity value of 60.2% and a specificity of 91.7%25 were obtained.[14]

The increase in NLR values in Covid-19 patients occurs due to the infection process of the Sars-COV-2 virus where neutrophils will be activated and degranulate, activated neutrophils will induce the release of Reactive Oxygen Species (ROS) such as superoxide radicals and H2O2 which lead to oxidative stress and contribute to the occurrence of cytokine storms. Another factor is the increase in neutrophils that can be triggered by inflammatory factors (IL-1, IL-6, TNF-α, IFN-γ) produced by lymphocytes. The main form of human defense against viruses depends mainly on lymphocytes, where T helper lymphocyte cells (CD4) and cytotoxic T lymphocytes (CD8) are the spearheads of virus resistance. However, in the process a lymphocyte exhaustion mechanism can occur in an unresponsive cell state with persistent antigenic T cell activation in lung epithelial cells. This causes T cells to be insensitive, shrink, and is accompanied by lymphocyte cell apoptosis. The presence of substances such as PD-1 and Tim-3 are biomarkers of lymphocyte exhaustion.[7] In addition, several other theories such as viruses can lyse lymphocytes directly, cytokine storm which causes lymphocyte apoptosis and lymphoid organ atrophy[15], mechanism of antibody dependent enhancement (ADE)[16], as well as lymphocyte dysfunction by the virus are the reasons why the NLR value is quite high in Covid-19 patients.[17] therefore, the neutrophil lymphocyte ratio during viral inflammation will increase. However, the concept of increasing NLR also applies to several other diseases and is not specific only for Covid-19 sufferers. Increased NLR can occur in septic patients, in cases of long-term use of corticosteroids,

acute cardiac infarction, and other respiratory infections so that NLR evaluation requires confirmation of clinical symptoms and other examination findings.[2,7]

CONCLUSION

In conclusion, this study validates that the Neutrophil-to-Lymphocyte Ratio (NLR) serves as a reliable and accessible alternative for the early diagnosis of COVID-19 in secondary healthcare facilities. The analysis demonstrated a moderate diagnostic accuracy with an AUC of 0.785 and a statistically significant p-value ($p < 0.05$; 95% CI 0.662–0.907). At the optimal cut-off point of ≥ 6.305, the NLR achieved a sensitivity of 70.17% and a specificity of 72.22%. It is important to note that comorbidities may act as confounding factors by elevating NLR levels in COVID-19 patients.

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