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The Combination of NLR and D-dimer as Predictor Instrument for the Severity of COVID-19

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ABSTRACT

Coronavirus Disease-19 (COVID-19) is caused by Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2). In severe cases, the immune response may cause a cytokine storm. Neutrophil Lymphocyte Ratio (NLR) and D-dimer are parameters that may be used to predict the severity of COVID-19. This study aims to determine the diagnostic validity of the combination of NLR and D-dimer on the severity of COVID-19 patients. The study population was hospitalized COVID-19 patients whose diagnosis were confirmed by real time-PCR. This was a retrospective cross-sectional study. The cut-off value was based on the Area Under Curve (AUC) of the Receiver Operator Characteristic Curve (ROC) analysis and the combination of NLR and D-Dimer validity was tested against the severe and non-severe COVID-19 groups by assessing sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), positive Likelihood Ratio (LR+) and negative Likelihood Ratio (LR-). There were 1,314 subjects. Seven hundred and forty-four were in the severe group, 570 in the non-severe group. The combination of NLR > 4.02 and D-dimer > 1.12 mg/L on the severity of COVID-19 showed a sensitivity value of 70.8%, specificity 98.3%, PPV 98.1%, NPV 72.1%, LR+ 40.38 and LR- 0.30. The combination of NLR >4.02 and D-dimer >1.12 mg/L for the severity of COVID-19 showed high specificity and PPV (98.3% and 98.1%). This was also supported by the LR+ value, which indicates that if NLR > 4.02 and D-dimer > 1.12 mg/L, it may cause severe COVID-19 by 40.38 times compared to NLR ≤ 4.02, and D-dimer ≤ 1.12 mg/L. The combination of NLR and D-Dimer can be used to predict the severity of COVID-19.

Keywords: COVID-19, D-dimer, NLR, severity

INTRODUCTION

Coronavirus Disease-19 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2), which was first found in Wuhan, China in December 2019. Within a short period, COVID-19 spread worldwide and was declared a global pandemic by World Health Organization (WHO) on March 11th, 2020.[1,2]

COVID-19 mainly spreads through the respiratory tract with various clinical manifestations, ranging from asymptomatic, and mild-moderate symptoms to severe symptoms such as pneumonia, sepsis, septic shock, and Acute Respiratory Distress Syndrome (ARDS) and Multiple Organ Dysfunction Syndrome (MODS).[3] In severe COVID-19, the immune response may occur excessively and may cause a cytokine storm, which affects hematopoiesis and hemostatic system significantly, and may trigger the incidence of Systemic Inflammatory Response Syndrome (SIRS).[4]

One of the physiologic responses of the body against systemic inflammation is marked by an increase in neutrophil count and a decrease in lymphocyte count. The Neutrophil Lymphocyte Ratio (NLR) is a simple, easy, and effective hematology examination to determine the degree of severity in COVID-19 patients. An increase in NLR reflects an increase in the inflammatory process, triggering the progressivity of COVID-19 and is associated with poor prognosis.[5-7] Liu *et al.*, stated that a NLR of ≥ 3.13 is associated with a poorer prognosis in COVID-19.[8,9]

Hyperinflammation occurring in COVID-19 causes an increase in the activation of the coagulation cascade and excessive thrombin production, leading to an increase in thrombosis and thromboembolic event risks in blood vessels, both veins or arteries.[10] D-dimer is a fibrin degradation product, produced during the process of blood clot degradation through fibrinolysis. An increase of D-dimer is frequently found in severe COVID-19 patients and is a predictor of ARDS, need for intensive care unit admission, and death.[11] A study by Zhou *et al.*, showed that increased D-dimer of >1.0 µL/mL is the strongest predictor of mortality in COVID-19 patients.[12]

Based on the study by Liu *et al.* and Zhou *et al.*, NLR and D-dimer examination may describe the

degree of severity and prognosis in COVID-19 patients. However, the use of combined NLR and D-dimer examination towards the degree of severity of COVID-19 has not been evaluated. This study aims to determine the validity of combined NLR and D-dimer examination as a diagnostic test towards the degree of severity in COVID-19 patients admitted at Dr. Hasan Sadikin Hospital, Bandung from March 2020 to February 2021.

METHODS

This was a diagnostic study with a cross-sectional design. Data were obtained retrospectively through medical records and Laboratory Information System at Dr. Hasan Sadikin Hospital (HCLAB, Sysmex, Asia Pacific). The population in this study were COVID-19 patients who were confirmed positive through real-time PCR within 48 hours since admission and were admitted in the COVID-19 isolation ward from March 2020 to February 2021. The sampling method for this study was total sampling, i.e., all populations that fulfilled inclusion criteria were enrolled as study subjects.

The inclusion criteria of this study were: Adult patients (aged ≥ 18 years); Categorized as symptomatic COVID-19 infection (mild, moderate, severe, or critical degree); Examined for NLR and D-dimer at the time of admission, or at least 48 hours after admission in the isolation ward. The exclusion criteria for this study were: Inaccessible medical records data at the time of the study in progress; Patients who have been admitted to intensive care or special isolation ward prior to confirmation of COVID-19 diagnosis.

The severity degree of COVID-19 was categorized based on WHO criteria and COVID-19 Prevention and Control Guideline, Ministry of the Health Republic of Indonesia (Pedoman Pencegahan dan Pengendalian COVID-19 Kemenkes RI). The degree of severity was divided into two categories, namely: Severe COVID-19, marked by SpO_2 of < 90%, respiratory rate of > 30 times/minute, and presence of respiratory distress signs. Also included into severe COVID-19 was critically ill COVID-19, marked by ARDS, sepsis, and septic shock; Non-severe COVID-19, were those who did not fulfill the criteria for severe and critical COVID-19.[13]

This study aims to determine the cut-off value based on Receiver Operator Characteristic (ROC) curve analysis in form of Area Under Curve (AUC) value to predict the COVID-19 degree of severity. Cut-off values from previous studies were also utilized i.e., NLR ≥ 3.13, based on the study by Liu *et al*., and cut-off value for D-dimer of >1.0 µg/mL based on the study by Zhou *et al*.[8,12] All data was documented using Microsoft Excel® software. Study subject characteristics were analyzed for COVID-19 degree of severity(severe/non-severe) using Chi-Square test via SPSS® ver. 19.0 program and was presented in a frequency distribution table. Diagnostic testing for combined NLR and D-dimer toward COVID-19 degree of severity, was analyzed to determine the sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Likelihood Ratio (LR). This study has been approved by Medical Research Ethical Committee, Dr. Hasan Sadikin Hospital Bandung (No.: LB.02.01/X.2.2.1/20175/2021).

RESULTS AND DISCUSSIONS

Based on the obtained data, there were a total of 2,261 COVID-19 patients admitted to Dr. Hasan Sadikin Hospital. Study subjects with complete

Table 1. Study subject characteristics

Characteristics	Total (n=1314)		Degree of Severity			
			Severe (n=744)		Non-Severe (n=570)	
	n	%	n	%	n	%
Gender						
Male	660	50.2	389	52.3	271	47.5
Female	654	49.8	355	47.7	299	52.5
Age (years old)						
18-25	106	8.1	66	8.9	40	7.0
26-35	211	16.1	105	14.1	106	18.6
36-45	244	18.6	111	14.9	133	23.3
46-55	269	20.5	139	18.7	130	22.8
56-65	286	21.8	175	23.5	111	19.5
>65	198	15.1	148	19.9	50	8.8
Clinical outcome						
Improved	1,134	86.3	570	76.6	564	98.9
Deceased	180	13.7	174	23.4	6	1.1

medical records data and fulfilled the inclusion criteria were 1,314 patients. The study subjects' characteristics were presented in Table 1.

There were 744 subjects categorized into the severe group and 570 subjects in the non-severe group. The severe group was dominated by male subjects (52.3%), while the non-severe group was dominated by female subjects (52.5%). The severe group was mostly from the 56-65 years age group (23.5%), while the non-severe group was mostly from the 36-45 years age group (23.3%). The mortality rate of the severe group was 23.4% while the non-severe group was 1.1% (Table 1).

Based on Table 2, the severe group was dominated by subjects with NLR ≥ 3.13 (87.5%) and D-dimer ≥ 1 µL/mL (91.8%). This result showed that the increase of NLR ≥ 3.13 and D-dimer ≥ 1 µL/mL was statistically significant in determining the severity degree of COVID-19 (p<0.001).

Based on ROC analysis, D-dimer had a higher AUC compared with NLR regarding the severity degree of COVID-19 (0.948 vs 0.850; p<0.001) in this study. The obtained cut-off value for NLR was >4.02 with a sensitivity of 79.6% and specificity of 76.3%; meanwhile, the cut-off value for D-dimer was >1.12 mg/L with a sensitivity of 88.8% and specificity of 89.5%. The receiver operator characteristic curve for NLR and D-dimer towards the COVID-19 degree of severity was presented in Figure 1.

Based on Table 3, the severe group was dominated by subjects with NLR of > 4.02 (79.6%) and a D-dimer value of >1.12 mg/L (88.8%). This result showed that increased NLR > 4.02 and D-dimer >1.12 mg/L were statistically significant towards an increase in the degree of severity of COVID-19 (p<0.001).

Table 2. NLR and D-dimer parameter characteristics based on cut-off value from a study by Liu *et al.* and Zhou *et al.*

Variable	Total n=1314		Degree of Severity				p-value
			Severe (n=744)		Non-Severe (n=570)		
	n	%	n	%	n	%	
NLR							
≥ 3.13	860	65.4	651	87.5	209	36.7	**<0.001***
< 3.13	454	34.6	93	12.5	361	63.3	
D-dimer (µL/mL)							
≥ 1	771	58.7	683	91.8	88	15.4	**<0.001***
< 1	543	41.3	61	8.2	482	84.6	
NLR and D-dimer (µL/mL)							
NLR ≥ 3.13 and D-dimer ≥ 1	642	48.8	602	80.8	40	7.0	**<0.001***
NLR <3.13 and/or d-Dimer <1	673	51.2	143	19.2	530	93.0	

Abbreviation: Asterisk (*) indicates that the analysis was conducted using a Chi-Square test

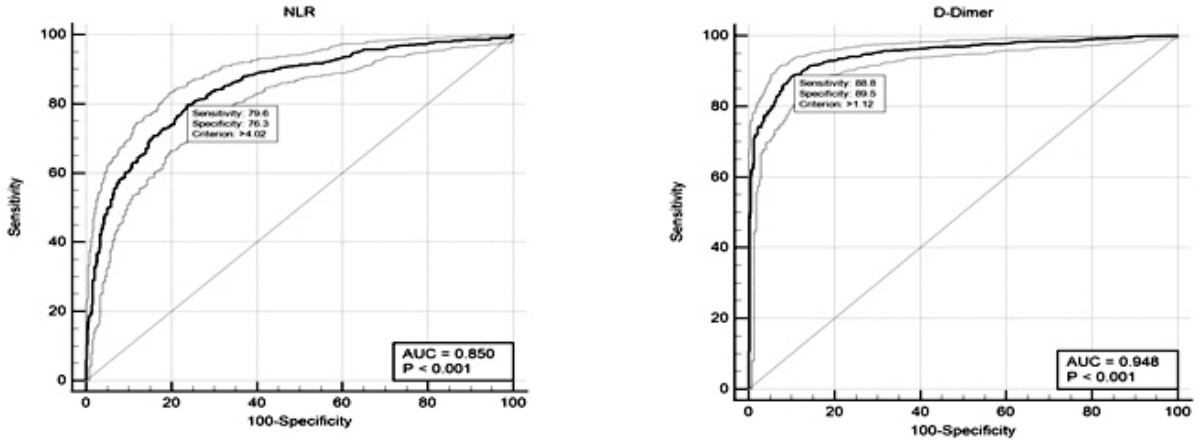

Figure 1. Receiver operator characteristic curve of NLR and D-dimer towards the degree of severity

Table 3. Parameter characteristics based on ROC cut-off value

Variable	Total n=1314		Degree of Severity				p-value
			Severe (n=744)		Non-Severe (n=570)		
	n	%	n	%	n	%	
NLR							
>4.02	860	65.4	592	79.6	135	23.7	**<0.001***
≤4.02	454	34.6	152	20.4	435	76.3	
D-dimer (mg/L)							
>1.12	771	58.7	661	88.8	60	10.5	**<0.001***
≤1.12	543	41.3	83	11.2	510	89.5	
NLR and D-dimer (mg/L)							
NLR >4.02 and D-dimer >1.12	642	48.8	527	70.8	10	1.8	**<0.001***
NLR ≤4.02 and/or D-dimer ≤1.12	673	51.2	217	29.2	560	98.2	

Abbreviation: Asterisk (*) indicates that the analysis was conducted using the Chi-Square test

Table 4. Combined NLR and D-dimer diagnostic test towards severity degree of COVID-19

Variable	Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR+ (95% CI)	LR- (95% CI)
NLR ≥ 3.13[8]	87.6	63.3	75.7	79.5	2.39 (2.14 – 2.67)	0.20 (0.16 – 0.24)
NLR > 4.02	79.6	76.3	81.4	74.1	3.36 (2.89 – 3.91)	0.27 (0.23 – 0.31)
D-dimer ≥ 1 µL/mL[12]	91.8	84.6	88.6	88.8	5.95 (4.90 – 7.22)	0.10 (0.08 – 0.12)
D-dimer >1.12 mg/L	88.8	89.5	91.7	86.0	8.44 (6.64 – 10.74)	0.13 (0.10 – 0.15)
NLR ≥ 3.13 and D-dimer ≥1 µL/mL[8.12]	80.8	93.0	93.8	78.8	11.52 (8.52 – 15.56)	0.21 (0.18 – 0.24)
NLR > 4.02 and D-dimer >1.12 mg/L	70.8	98.3	98.1	72.1	40.38 (21.81 –74.76)	0.30 (0.27 – 0.33)

Abbreviation: Sn: sensitivity, Sp: specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR+: positive Likelihood Ratio, LR-: negative Likelihood Ratio, CI: Confidence Interval

Table 4 showed that the diagnostic testing for combined NLR ≥ 3.13 and D-Dimer ≥ 1 µL/mL toward COVID-19 degree of severity, yielded high specificity and positive predictive value (93% and 93.8%, respectively). These results were supported by the LR+ value, which meant that the NLR value of ≥ 3.13 and D-dimer value of ≥ 1 µL/mL might oppose the possibility of severe COVID-19 11.52-fold higher than the NLR value of <3.13 and D-dimer value of <1 mg/L. Meanwhile, diagnostic testing for combined NLR > 4.02 and D-dimer > 1.12 µL/mL toward COVID-19 degree of severity, yielded high specificity and positive predictive value (98.3% and 98.1%, respectively). This result was supported by the LR+ value, which meant that the NLR value of > 4.02 and D-dimer value of >1.12 µL/mL might oppose the possibility of severe COVID-19 40.38-fold higher than the NLR value of ≤ 4.02 and D-dimer value of ≤ 1.12 mg/L.

Coronavirus Disease-19 is an infectious disease caused by SARS-CoV-2, which spread rapidly and became a global pandemic.[1,2] In severe cases, the immune response may occur excessively and may cause systemic cytokine storms, which trigger the incidence of SIRS, endotheliopathy, and hypercoagulation, which eventually leads to thrombosis.[4]

Hematology examinations, such as the NLR may be utilized to determine the degree of inflammation. An increase in NLR reflects an increase in the inflammatory process and is associated with a poor prognosis.[5-7] During inflammation, neutrophils are the main component of leukocytes, which actively migrates toward the immune system or organs. Neutrophils release Reactive Oxygen Species (ROS) in massive amounts that induce DNA cell damage and cause the virus to freely exit the cell. Antibody-Dependent Cell-mediated Cells (ADCC) can eliminate viruses directly and trigger humoral immunity. Neutrophils were induced by virus-associated inflammatory factors, such as IL-6, IL-8, TNF-α, granulocyte colony-stimulating factor, and interferon-gamma factors, which were produced by lymphocytes and endothelial cells. The virus-induced immune response is dependent on lymphocytes. Systemic inflammation will significantly suppress cellular immunity; thus, reducing $CD4^+$ T lymphocytes and increase $CD8^+$ suppressor T lymphocytes. Hence, virus-induced inflammation will increase NLR.[8] As stated in a study

by Liu *et al*. COVID-19 has a better prognosis if the NLR was < 3.13 and a poorer prognosis if the NLR was ≥ 3.13.[8]

The COVID-19 degree of severity could also be evaluated by conducting a hemostasis examination. In COVID-19, endothelial damage occurs and may activate platelets, causing hypercoagulation. This event resulted in fibrin deposition, an increase in D-dimer, and may cause the incidence of Disseminated Intravascular Coagulation (DIC). D-dimer is a fibrin degradation product produced during blood clot degradation via fibrinolysis, which became a marker for suspicion of thrombosis. An increase in D-dimer is frequently found in severe COVID-19 patients and is a predictor of incidence of ARDS. An increase in D-dimer is also associated with needs for intensive care unit admission and even death.[4] In their study, Zhou *et al*. showed that an increase of D-dimer > 1.0 µg/mL was the strongest predictor of mortality in COVID-19 patients. Meanwhile, Cui *et al.* stated that a D-dimer of >1.5 µg/mL was a predictor for venous thromboembolism in COVID-19 patients with a sensitivity of 85% and specificity of 88.5%. Thus, D-dimer was one of the modalities that may evaluate the coagulation and fibrinolysis process in COVID-19 patients.[14]

In this study, diagnostic testing for combined NLR of ≥ 3.13 and D-dimer of ≥ 1 µL/mL toward COVID-19 degree of severity based on the cut-off value from studies by Liu *et al*. and Zhou *et al*. yielded high specificity and positive predictive value (93% and 93.8%, respectively). This result was supported by the LR+ value, which meant that the NLR value of ≥ 3.13 and D-dimer value of ≥ 1 µL/mL might oppose the possibility of severe COVID-19 11.52-fold higher than the NLR value of < 3.13 and D-dimer value of < 1 mg/L. Meanwhile, diagnostic testing for combined NLR > 4.02 and D-dimer > 1.12 µL/mL towards severity degree of COVID-19 based on ROC cut-off value yielded a better specificity and PPN (98.3% and 98.1%, respectively). This result was supported by LR+ value, which meant that the NLR value of > 4.02 and D-dimer value of >1.12 µL/mL might oppose the possibility of severe COVID-19 40.38-fold higher than the NLR value of ≤ 4.02 and D-dimer value of ≤1.12 mg/L.

These results were in accordance with study results by Laguna *et al*., which stated that increased NLR reflected the inflammation process and lead to a poor prognosis.[5] Yang *et al*. also stated that among patients aged ≥ 49.5 years with NLR of ≥ 3.3, there were 46.1% of mild COVID-19 patients would develop severe COVID-19 symptoms.[7] Ciu *et al.* in their study also stated that an increase of D-dimer >1.5 µg/mL was a predictor for the thromboembolic event in COVID-19 with a sensitivity of 85% and specificity of 88.5%.[14]

Among 570 subjects in this study with severe disease improved and recovered. This might be due to the adequate immune and therapeutic response of the subjects. However, there were 6 subjects with the non-severe disease who died. This might be due to the poor immune and therapeutic response of the subjects. This condition could also be due to the presence of comorbidities, secondary infection, or other inflammation, which were not documented in this study but might confound the clinical outcome of the patient. The limitation of this study was the lack of detailed data exploration of the patient's comorbidities.

CONCLUSIONS AND SUGGESTIONS

Diagnostic testing for combined NLR of > 4.02 and D-dimer of > 1.12 mg/L yielded a good specificity and PPV towards the severity degree of COVID-19 (98.3% and 98.1%, respectively), and might oppose severe COVID-19 risk of 40.38-fold higher compared with NLR of ≤ 4.02 and D-dimer of ≤ 1.12 mg/L within the first 48 hours of admission.

Further studies to trace the presence of comorbidities of study subjects in detail. The result of this study could be helpful for clinicians to raise awareness regarding patient conditions within the first 48 hours of admission, to plan COVID-19 treatment strategies and provide a better prognosis for the patients.

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