INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

2022 March; 28(2): 111-114
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Correlation between Derived Neutrophil to Lymphocyte Ratio and D-Dimer in COVID-19 Patients

Natra Dias Surohadi, Ria Triwardhani

Department of Clinical Pathology, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia. E-mail: dokternatra@gmail.com

ABSTRACT

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). Several studies suggest that the derived Neutrophil to Lymphocyte Ratio (d-NLR) and D-dimer are inflammatory markers in various diseases and can be used to monitor prognosis and mortality. This study was conducted to find the correlation between d-NLR and D-dimer in COVID-19 patients. The results were expected to be inflammatory markers and a predictor for patients with clinical deterioration to avoid the risk of death. This study used a retrospective observational method with a cross-sectional approach at Dr. Kariadi Hospital, Semarang using secondary data involving confirmed COVID-19 respondents from March to August 2020. The Spearman test was used to analyze data, and $p < 0.05$ was stated significantly. Thirty-three respondents with confirmed COVID-19 results, the median value of d-NLR 6.14 (3.55-15.67) and median value of D-dimer 7110 (2460-21770) mg/L were tested for the correlation. Spearman correlation test showed $p=0.046$; and $r=0.350$. Increased d-NLR and D-dimer levels, known as systemic inflammatory response markers, were reported in COVID-19. These increased levels were related to the severity of the COVID-19 disease. There was a highly significant positive correlation between d-NLR and D-dimer in COVID-19 patients.

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

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). SARS CoV-2 is a new type of coronavirus that has not been previously identified in humans. Two types of Coronaviruses are known to cause diseases and severe symptoms, such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). Common signs and symptoms of COVID-19 infection include symptoms of acute respiratory distress such as fever, cough, and shortness of breath. The average incubation period is 5-6 days, with the most extended period being 14 days. Severe cases of COVID-19 can cause pneumonia, acute respiratory syndrome, kidney failure, and even death.[1]

On December 31, 2019, the Official WHO representative in China reported a case of pneumonia of unknown etiology in Wuhan City, Hubei Province, China. On January 7, 2020, China identified the case as a new type of Coronavirus. On January 30, 2020, WHO declared the incident a Public Health Emergency of World Concern (KKMMD). Later on, on March 11, 2020, WHO announced COVID-19 as a pandemic.[1,2]

The number of cases kept increasing very quickly and spread to various countries in a short time. As of July 9, 2020, WHO reported 11,84,226 confirmed cases with 545,481 deaths worldwide (Case Fatality Rate/CFR 4.6%). Indonesia declared its first case on March 2, 2020. Chances are increasing and spreading rapidly throughout Indonesia. As of July 9, 2020, the Ministry of Health reported 70,736 confirmed cases of COVID-19 with 3,417 deaths (CFR 4.8%).[1]

Inflammation caused by the transmission of infectious diseases can play an essential role in developing the COVID-19 virus. The severe inflammatory response contributes to a weak adaptive immune response, resulting in an imbalanced immune response. Interactions with blood cells are very important in the pathophysiology of inflammation, immune response, hemostasis, and oncogenesis.[1] It has been stated that the derived Neutrophil to Lymphocyte Ratio (d-NLR) and D-dimer are inflammatory markers in various diseases and can be used to monitor prognosis and mortality.

METHODS

This research was an analytic observational study with a cross-sectional approach conducted at Dr. Kariadi Hospital, Semarang, from March to August 2020. The study sample was COVID-19 patients who were treated in the inpatient and intensive care units of Dr. Kariadi Hospital, Semarang. Patients who were confirmed positive for COVID-19 with positive swab/PCR results, male and female, with ages ranging from 20 to 75 years, were included in this study. Pregnant patients and children, patients with a history of malignancy, patients undergoing radiation/chemo, and patients with rheumatoid arthritis and liver disease were excluded from this study. Research permission was obtained with ethical approval from the Medical and Health Research Ethics Committee of Dr. Kariadi Hospital, Semarang with number 639/EC/KEPK-RSDK/2020.

The data collected in this study were the d-NLR value calculated with the formula d-NLR = ANC/(WBC–ANC) and cut-off = 2.8.2 and D-dimer with cut-off > 2.14 mg/L3. Data were then processed using the IBM SPSS Statistics program version 25. Shapiro-Wilk test was used to determine the normality of data. It was found that data of d-NLR and D-dimer were not normally distributed; therefore, the Spearman rank test was used to determine the correlation between d-NLR and D-dimer in COVID-19 patients. p < 0.05 was stated significant.

RESULTS AND DISCUSSIONS

A total of 33 COVID-19 subjects participated in the study. The distribution of the characteristics of the subjects is presented in Table 1. There were a higher number of male (75.8%) than female subjects (24.2%). The mean age was 54 years.

Table 1. Characteristics of research subjects

Variable (n=29)	Mean±SD	Median (min–max)
Gender	Male 25 (75.8%) Female 13 (24.2%)	
Age (years)	50.88±13,654	54 (22-73)
Absolute neutrophils count (/uL)	14355,85±6327,795	13630 (4374-29348)
Leukocytes (x10^3/uL)	16,467±6,9304	15 (5.4-31.9)
Neutrophils (%)	86.64±4.703	86 (78-94)
d-NLR*	7,6706±3,69977	6.14 (3.55-15.67)
D-dimer (mg/L)*	9427,82±6399,106	7110 (2460-21770)

Note: SD (Standard Deviation); min (minimum); max (maximum), *Abnormal distribution, d-NLR, derived Neutrophils to Lymphocyte Ratio

Data of d-NLR and D-dimer were analyzed. First, the shapiro-Wilk test was used to determine the normality of data. Because the data were not normally distributed, the Spearman Rank test was then used. The relationship between d-NLR levels and D-dimer can be seen in Table 2.

Table 2. Correlation between d-NLR and D-dimer

Variable	D-dimer (mg/L)	
	p	r
Absolute neutrophils count (/uL)	0.214	0.222
Leukocytes (x10^3/uL)	0.289	0.190
Neutrophils (%)	0.046*	0.350
d-NLR	0.046*	0.350

Spearman Rank test, *p< 0.05; r, correlation coefficient

There was a weak positive correlation between d-NLR and D-dimer (p=0.046; and r=0.350). The distribution of d-NLR data with D-dimer can be seen in Figure 1.

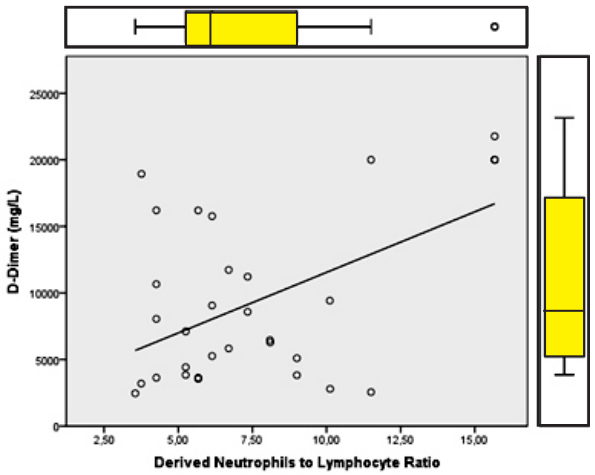

Figure 1. Scatter plot of correlation between d-NLR and D-dimer in COVID-19 patients

Both d-NLR and D-dimer levels, known as systemic inflammatory markers, were found to increase in this study. This result was similar to the previous study conducted by Yang *et al*., which reported an increased d-NLR in COVID-19 cases with an AUC of 0.841.2 Droplets spread SARS CoV-2 through coughing, sneezing, or direct contact with an infected individual.[3] The virus enters the mucous membranes of host cells, and travels through the respiratory tract and into the alveoli in the lungs. The virus binds specifically to the Angiotensin-Converting Enzyme 2 (ACE2) receptor residing on type II pneumocytes in the alveoli, and viral replication occurs. Damaged type II pneumocytes will stimulate macrophages, monocytes, and neutrophils to the site of infection to secrete proinflammatory cytokines such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Tissue Necrosis Factor-Alpha (TNF-α) and chemokines such as CCL2 and CXCL8.[4]

Derived NLR has recently been recognized as a marker of the systemic inflammatory response as a modified form of Neutrophil Lymphocyte Ratio (NLR).[5]

D-dimer is a specific antigen resulting from the degradation of factor XIIIa bound to fibrin. Specific monoclonal antibodies for D-dimer antigens were developed to help clinicians distinguish the products of fibrinogen and fibrin degradation through laboratory tests. As measured in clinical samples, antigen D-dimer results from the degradation of fibrin formed by the combined action of thrombin, factor XIIIa, and plasmin.[6]

D-dimer represents activation of coagulation and fibrinolysis. COVID-19 cases are associated with coagulation abnormalities, and D-dimer values have been reported to increase in death cases due to COVID-19.[7]

Several abnormal hematological parameters in COVID-19 patients were lymphopenia, neutrophilia, elevated D-dimer, and fibrinogen levels.[8-11] However, the clinical implications of these parameters remain unclear. The NLR is an index that is easily calculated from a complete blood count. In addition, many studies have shown that the NLR has prognostic value in many conditions, including sepsis, cardiovascular disease, tumor malignancy, etc.[12-15] Derived neutrophil to lymphocyte ratio, a modification of the NLR, is also used as a marker of systemic inflammation and prognosis in several types of cancer such as gastrointestinal and breast cancer.[2] Increased thrombogenicity and increased platelet aggregation have been found in community-acquired pneumonia. It was recently reported that COVID-19 could induce massive prothrombotic status.[16,17] D-dimer was found to be associated with the prognosis of novel influenza A (H1N1) pneumonia 2009.[18]

Coagulation disorders often occur in severe cases of COVID-19 patients, which are generally characterized by prolonged Prothrombin Time (PT) and increased D-dimer. According to research by Tang *et al*., abnormal coagulation, such as increased D-dimer, more prolonged PT, and partial thromboplastin time were found in COVID-19 patients who died compared to those who survived.[19,20]

This study showed a weak positive correlation between d-NLR and D-dimer levels with p-value=0.046; and r=0.350. This finding indicated that the more severe inflammation would increase the d-NLR value and D-dimer in COVID-19 patients.

No explanation of other factors affecting d-NLR and D-dimer remained the limitation of this study. In addition, there was no classification of patients based on the disease severity in this study, leading to the possibility of selection bias.

CONCLUSIONS AND SUGGESTIONS

This study found a weak positive significant relationship between d-NLR and D-dimer in COVID-19 patients. It was suggested to perform a further analysis that focuses on other factors affecting the value of d-NLR and D-dimer. It was expected that additional researchers could expand the sample's characteristics or use samples in other areas by collecting a more representative number of samples.

REFERENCES

1. Kementerian Kesehatan Republik Indonesia. Pedoman pencegahan dan pengendalian Coronavirus Disease (COVID-19). Revisi ke-5. Jakarta, Kementerian Kesehatan RI, 2020; 1-214.
2. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR, and PLR in COVID-19 patients. International Immunopharmacology, 2020; 84: 106504.
3. Yao Y, Cao J, Wang Q, Shi Q, Liu K, *et al*. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: A case-control study. Journal of Intensive Care, 2020; 8: 49.
4. Hussain A, Kaler J, Tabrez E, Tabrez S, Tabrez Shams. Novel COVID-19: A comprehensive review of transmission, manifestation, and pathogenesis. Cureus, 2020; 12(5): 1-14.
5. Esklizmir G, Uz U, Onur E, Ozyurt B, Cikrikci GK, *et al*. The evaluation of pretreatment neutrophil-lymphocyte ratio and derived netrophil-lymphocyte ratio in

patients with laryngeal neoplasms. Braz J Otorhinolaryngol, 2019; 85(5): 578-587.
6. Greenberg CS. The role of D-dimer testing in clinical hematology and oncology, clinical advances in hematology & oncology. Clinical Advances in Hematology and Oncology, 2017; 15: 580-583.
7. Zhang L, Yan X, Fab Q, Liu H, Liu X, *et al.* D-dimer levels on admission to predict in-hospital mortality in patients with COVID-19. J Thromb Haemost, 2020; 18: 1324–1329.
8. Guan W, Ni Z, Hu Y, Liang W, Ou C, *et al*. Clinical characteristics of Coronavirus disease 2019 in China. New Engl J Med, 2020; 382: 1708-20.
9. Huang C, Wang Y, Li X, Ren L, Zhao J, *et al*. Clinical features of patients infected with 2019 novel Coronavirus in Wuhan, China. The Lancet, 2020; 395(10223): 497-506.
10. Chen N, Zhou M, Dong X, Qu J, Gong F, *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel Coronavirus pneumonia in Wuhan, China: A descriptive study. The Lancet, 2020; 395(10223): 507-513.
11. Fan BE, Chong V, Chan S, Lim GH, Lim K, *et al.* Hematologic parameters in patients with COVID-19 infection. Am J Hematol, 2020; 95(6): E131-E134.
12. Russell CD, Parajuli A, Gale HJ, Bulteel NS, Schuetz P, *et al*. The utility of peripheral blood leucocyte ratios as biomarkers in infectious diseases: A systematic review and meta-analysis. J Infect, 2019; 78(5): 339-348.
13. Huguet E, Maccallini G, Pardini P, Hidalgo M, Obregon S, *et al.* Reference values for Neutrophil to Lymphocyte Ratio (NLR), a biomarker of cardiovascular risk, according to age and sex in a Latin American population. Curr Probl Cardiol, 2019; 100422.
14. Park JS, Seo KW, Choi BJ, Choi SY, Yoon MH, *et al*. Importance of prognostic value of neutrophil to lymphocyte ratio in patients with ST-elevation myocardial infarction. Medicine (Baltimore), 2018; 97(48): e13471.
15. Mei Z, Shi L, Wang B, Yang J, Xiao Z, *et al*. Prognostic role of pretreatment blood neutrophil to lymphocyte ratio in advanced cancer survivors: A systematic review and meta-analysis of 66 cohort studies. Cancer Treat Rev, 2017; 58: 1-13.
16. Violi F, Cammisotto V, Pignatelli. P. Thrombosis in COVID-19 and non-COVID-19 pneumonia: Role of platelets. Platelets, 2021; 1–9.
17. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, *et al*. Coagulopathy and antiphospholipid antibodies in patients with COVID-19. New Engl J Med, 2020; 382(17): e38.
18. Fu J, Kong J, Wang W, Wu M, Yao L, *et al*. The clinical implication of dynamic neutrophil to lymphocyte ratio and D-dimer in COVID-19: A retrospective study in Suzhou China. Elsevier Thrombosis Research, 2020; 192: 3–8.
19. Soeroto AY, Santoso P, Pranggono EH, Kulsum ID, Pitoyo CW, *et al.* Review article khusus kompendium diagnostik dan pengobatan COVID-19 (INTERIM) Perhimpunan Respirologi Indonesia (PERPARI), 2020; 7(1): 17–49.
20. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel Coronavirus pneumonia. J Thromb Haemost, 2020; 18(4): 844–7.