Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Feb 22; 10(B):335-338.
https://doi.org/10.3889/oamjms.2022.8545
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Hematology*

The Correlation between Neutrophil-to-Lymphocyte Ratio with C-reactive Protein and D-dimer Level among Indonesian COVID-19 Cases

Linda Rotty[1]*, Jerome Kurube[2], Paulus Novian Harijanto[3], Frans Wantania[4], Harlinda Haroen[1], Cecilia Hendratta[1], Pearla Lasut[1], Christian Kawengian[1], Randy Adiwinata[2]

[1]Department of Internal Medicine, Division of Hematology and Medical Oncology, Faculty of Medicine, Universitas Sam Ratulangi, Prof. dr. R. D. Kandou Hospital, Manado, Indonesia; [2]Department of Internal Medicine, Faculty of Medicine, Universitas Sam Ratulangi, Prof. dr. R. D. Kandou Hospital, Manado, Indonesia; [3]Department of Internal Medicine, Division of Tropical and Infectious Disease, Faculty of Medicine, Universitas Sam Ratulangi, Prof. dr. R. D. Kandou Hospital, Manado, Indonesia; [4]Department of Internal Medicine, Division of Cardiology, Faculty of Medicine, Universitas Sam Ratulangi, Prof. dr. R. D. Kandou Hospital, Manado, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Rotty L, Kurube J, Harijanto PN, Wantania F, Haroen H, Hendratta C, Lasut P, Kawengian C, Adiwinata R. The Correlation between Neutrophil-to-Lymphocyte Ratio with C-reactive Protein and D-dimer Level among Indonesian COVID-19 Cases. Open Access Maced J Med Sci. 2022 Feb 22; 10(B):335-338. https://doi.org/10.3889/oamjms.2022.8545
Keywords: Coronavirus disease-19; C-Reactive Protein; D-dimer; Neutrophil-to-lymphocyte ratio; Inflammation
***Correspondence:** Linda Rotty, Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Sam Ratulangi/Prof. dr. R. D. Kandou Hospital, Manado, Indonesia. Phone: +62-431-7283949. Email: linda_rotty@yahoo.com
Received: 07-Jan-2022
Revised: 30-Jan-2022
Accepted: 14-Feb-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Coronavirus disease-19 (COVID-19) pandemic has resulted high number of mortalities globally. Several inflammatory and coagulation biomarkers have been studied for predicting and differentiating severe COVID-19 such as C-reactive protein (CRP) and D-dimer. However, those markers may not readily available in developing countries.

AIM: The aim of the study was to assess the utility of neutrophil-to-lymphocyte ratio (NLR), a widely available and inexpensive laboratory examination, as reliable inflammatory biomarkers for Indonesian COVID-19 patients; by analyzing the correlation of NLR level with CRP and D-dimer plasma level.

METHODS: We conducted cross-sectional study in Professor Dr. R.D. Kandou Hospital, Manado involving RT-PCR confirmed and hospitalized COVID-19 patients. Lymphocyte count, NLR, CRP, and D-dimer were examined in severe and non-severe COVID-19 cases at hospital admission. Correlation test was done using Spearman correlation test.

RESULTS: A total of 40 COVID-19 patients were included in the analysis, with 50% having mild disease and other half having severe disease. The NLR, CRP, and D-dimer were significantly higher in severe COVID-19 group. Significant correlation was found between NLR and CRP ($p = 0.001$ and $r = 0.506$) and also with D-dimer level ($p = 0.000$ and $r = 0.570$) in differentiating severity of COVID-19.

CONCLUSION: NLR is correlated with CRP and D-dimer level; therefore, NLR may serve as reliable, cost-effective, and practical inflammatory biomarker for differentiating severe and non-severe COVID-19 cases.

Introduction

Coronavirus disease-19 (COVID-19) is an ongoing global pandemic which has resulted over 273 million infected cases and more than 5.3 million deaths according to the World Health Organization (WHO) as of December 19, 2021 [1]. The number of new and death cases is predicted to be still fluctuating due to the incoming of new COVID-19 variants, weaning of protective antibody titer, and other factors. Unfortunately, until now, there are still no specific anti-SARS-CoV-2 drugs. The most of COVID-19 death cases were attributed to multiple organ failure due to cytokine storm and pulmonary embolism [2]. Several efforts have been employed to reduce those fatally COVID-19 complications, such as the use of dexamethasone and anticoagulant prophylaxis for hospitalized COVID-19 patients [3]. The evidence of cytokine storm presence in COVID-19 was proved by many studies which shown by high inflammatory cytokines and biomarkers such as interleukin-6 (IL-6), interferon (IFN)-α, IFN-γ, C-reactive protein (CRP), and others. C-reactive protein has been shown as reliable early biomarker to predict risk for severity of COVID-19 [4], [5]. Therefore, patients with the high level of CRP level on admission may be placed for closer observation. D-dimer is widely known as marker of inflammation and presence of thrombus. In COVID-19 cases, elevated D-dimer was associated with more severe COVID-19 disease course and higher risk of mortality [6]. While CRP and D-dimer are becoming an established COVID-19 prognostic biomarkers, those biomarkers examination may not be applicable in low- and middle-income countries or in limited resources health-care facilities. A cost-effective yet readily available and accurate biomarker is needed. Therefore, we would like to assess the utility of neutrophil-to-lymphocyte ratio (NLR),

a simple, widely available, and inexpensive laboratory examination, as reliable inflammatory biomarkers for Indonesian COVID-19 patients; by comparing NLR level with CRP and D-dimer plasma level [7].

Methods

We conducted a cross-sectional study in Professor. Dr. R. D. Kandou Hospital, Manado, North Sulawesi, Indonesia and recruited confirmed mild and severe hospitalized COVID-19 patients aged older than 18 years old from June to August 2020. We excluded patients with waist circumference below 90 cm, patients with cancer, Human Immunodeficiency Virus (HIV) infection, and autoimmune disease. The disease severity was defined using National Guidelines of COVID-19 Management [8]. Mild case was defined as COVID-19 patients with the upper respiratory tract infection symptoms, without pneumonia and the need for oxygen supplementation. Severe case was defined as severe pneumonia which reflected by fever, cough, dyspnea, having respiratory rate more than 30 times/min, severe respiratory distress, and oxygen saturation below 90%. Confirmation of COVID-19 was based on Real-Time-Polymerase-Chain-Reaction (RT-PCR) testing. Informed consent was taken before the study and samples were collected with consecutive sampling methods. Sera were collected on the admission day. Each patient's demographic, clinical, and hematological data were recorded. The NLR was calculated by dividing the number of neutrophile to lymphocyte count. The CRP value was counted using immunoturbidimetric method. Normal CRP value was defined as lower than 5 mg/dl. D-dimer level was quantified using Enzyme-linked Fluorescent Immunoassay (ELFA). D-dimer count was normal if below 0.5 μg/ml.

Data analysis was supported with Statistical Package for the Social Science (SPSS) version 22. Continuous variables were denoted as mean ± standard deviation. Normality test was conducted using Shapiro–Wilk test. Unpaired t-test or Mann–Whitney test was used to compare the mean of NLR, D-dimer, and CRP between mild and severe COVID-19 group. Pearson or Spearman correlation test was used to determine the correlation between NLR and D-dimer and CRP value.

Results

We recruited 40 COVID-19 patients during study period with half of them were having severe disease and 50% were male. The mean age of patients was 53.53 ± 15.15 years old. According to the disease severity, we found that 20 patients were having mild disease and 20 patients were having severe COVID-19 disease. The overall mean of absolute lymphocyte count was 1790.87 ± 1018.78 cells/mm^3. The overall mean of NLR was 7.38 ± 6.94. The overall mean of CRP and D-dimer was 23.73 ± 19.39 mg/dl and 4.88 ± 6.36 μg/ml, respectively. Detail regarding our subject's characteristics and comparison between mild and severe COVID-19 group can be seen in Table 1.

In our study, the Mann–Whitney test showed that NLR value was significantly higher in severe COVID-19 disease group compared to mild COVID-19 group with p value of 0.002. Similar finding was also found in analysis of CRP (p = 0.000) and D-dimer (p = 0.001). The Spearman correlation test showed significant correlation between NLR and CRP level (p = 0.001) with correlation coefficient of 0.506. The correlation analysis also showed significant correlation (p = 0.000) between NLR and D-dimer level correlation coefficient of 0.570.

Discussion

The NLR role as prognostic marker for COVID-19 patients was extensively studied. NLR may become a reliable, accessible, and cost-effective inflammation parameters, especially in developing country such Indonesia during COVID-19 pandemic era. NLR was easily calculated at emergency department using routine laboratory test [9], [10]. A meta-analysis by Simadibrata *et al.* which involving more than 5000 COVID-19 patients found that the higher NLR levels on admission were associated with 2.7 times higher mortality risk compared to patients with normal NLR. The higher NLR level was also associated with more severe disease COVID-19 course [7]. This finding was in line with our result which the higher NLR value is more commonly found in severe COVID-19 group. Elevation of NLR value may be explained by the increased level of neutrophil due to inflammation response and due to the lymphocytopenia. Lymphocytopenia occurred in 90% of severe COVID-19 cases [11], [12], [13]. Systematic review by Zhao *et al*. concluded that COVID-19 patients with lymphocytopenia are associated with nearly 3 times increased risk for severe disease course [12]. Several mechanisms have been proposed regarding the occurrence of lymphocytopenia in COVID-19 patients such as SARS-CoV-2 may induce apoptosis and pyroptosis of lymphocytes, bone marrow suppression due to proinflammatory cytokines released, thymus suppression, activation-induced cell death of lymphocytes, tissue redistribution of lymphocytes, and several other pathways [11].

In our study, we found significant correlation with moderate strength between NLR and CRP value. The CRP which determined as acute phase reactant plasma

Table 1: Subject's characteristic and comparison between mild and severe COVID-19 group

Characteristics	All (n = 40)	Mild (n = 20)	Severe (n = 20)	p value*
Age, mean ± SD	53.53 ± 15.15	51.54 ± 14.77	55.54 ± 17.86	0.977
Male, n (%)	23 (57.5)	14 (58.3)	9 (45)	0.314
Comorbidity				
Hypertension, n (%)	21 (52.5)	11 (55)	10 (50)	0.141
Diabetes, n (%)	12 (30)	5 (25)	7 (35)	0.377
Coronary Artery Disease, n (%)	6 (15)	2 (10)	4 (20)	0.160
Hyperuricemia, n (%)	3 (7.5)	1 (5)	2 (10)	0.974
Hemoglobin, mean ± SD	12.6 ± 2.2	12.2 ± 2.1	13.0 ± 2.3	0.525
White Blood Cell, mean ± SD	14,002.5 ± 9,100.2	10,258.3 ± 4406.1	17,746.7 ± 13794.3	0.019
Platelet, mean ± SD	254,622.5 ± 136,593.4	297,625 ± 138,314.3	211,620 ± 134,872.5	0.897
Absolute lymphocyte count, mean ± SD	1,790.87 ± 1018.78	1,838.25 ± 800.67	1,743.49 ± 436.22	0.120
NLR, mean ± SD	7.38 ± 6.94	4.83 ± 2.93	9.93 ± 7.94	0.002
CRP, mean ± SD	23.73 ± 19.39	13.25 ± 15.01	34.21 ± 16.78	0.000
D-dimer, mean ± SD	4.88 ± 6.36	1.83 ± 1.45	7.93 ± 5.11	0.001

*Mann–Whitney test and Chi-square test were performed between mild and severe COVID-19 group to determine the p-value.

protein and mainly produced in liver was induced by several inflammatory cytokines such as IL-6. The use of CRP as inflammation biomarker has been established. The CRP value may decrease gradually over 18–20 h after inflammatory stimuli were stopped [14].The use of CRP in COVID-19 case management has been proposed as early marker for predicting severe COVID-19. Aji found that CRP threshold value of 26.9 mg/L may serve as optimal cutoff and every one-unit increase in CRP may lead to 5% increased risk for getting severe COVID-19 [5]. Smilowitz *et al*. found that elevated CRP level was associated with worse clinical outcome of COVID-19 which defined as more critical illness, higher in-hospital mortality, venous thrombo-embolism, acute kidney injury rate [15]. We also found higher CRP value in severe COVID-19 group in our study. We also found that NLR was correlated with CRP value which associated with more severe COVID-19 at higher level plasma level. Similar finding was also found by Mousavi-Nasab *et al*., which NLR was positively correlated with CRP value (r=0.23) [16]. Study in Indonesia conducted by Sukrisman *et al*. also showed strong correlation between NLR and CRP ($p < 0.001$, $r = 0.738$) [17]. All these findings showed that NLR may serve as suitable inflammation marker in remote area for predicting severe COVID-19.

Mortality in COVID-19 cases may attribute due to venous thromboembolism events caused by hypercoagulability condition. D-dimer value is regarded as hemostasis activation and thrombosis marker. Meta-analysis by Zhan *et al*. showed that D-dimer may predict severe cases of COVID-19 with moderate accuracy [18]. Another systematic review by Rostami *et al*. also found that increased 3–4 fold of D-dimer level was linked to poor prognosis and they recommended to measure D-dimer level in COVID-19 patients [19]. Poudel *et al*. proposed that D-dimer cutoff of 1.5 μg/ml on admission is the optimal cutoff for predicting mortality in COVID-19 cases [20]. In this study, we found significantly higher D-dimer level in severe COVID-19 group. Isbaniah *et al*. found that NLR and D-dimer value on admission was important for predicting clinical outcome of Indonesian COVID-19 patients [21]. Man *et al*. found that NLR was significantly correlated with D-dimer in predicting COVID-19 severity ($p < 0.001$ and $r = 0.49$) [22]. This finding is similar to our finding.

The limitation of our study is regarding the small number of subjects; therefore, further study with larger sample size is warranted to establish stronger correlation between NLR, CRP, and D-dimer level among Indonesia COVID-19 patients.

Conclusion

NLR was found well correlated with established inflammatory and coagulation marker which capable in predicting severe COVID-19. Therefore, NLR which easily calculated at emergency department using routine laboratory test even in remote area may serve as practical and cost-effective marker for guiding the physician in awareness regarding the need for intensive care.

References

1. World Health Organization. COVID-19 Weekly Epidemiological Update. Geneva: World Health Organization; 2021. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---21-december-2021 [Last accessed on 2021 Dec 23].
2. Jose RJ, Manuel A. COVID-19 cytokine storm: The interplay between inflammation and coagulation. Lancet Respir Med. 2020;8(6):e46-7. https://doi.org/10.1016/S2213-2600(20)30216-2
   PMid:32353251
3. World Health Organization. Living Guidance for Clinical Management of COVID-19. Geneva: World Health Organization; 2021 Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2 [Last accessed on 2021 Dec 23].
4. Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine storm in COVID-19: The current evidence and treatment strategies. Front Immunol. 2020;11:1708. https://doi.org/10.3389/fimmu.2020.01708
   PMid:32754163
5. Ali N. Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. J Med Virol. 2020;92(11):2409-11. https://doi.org/10.1002/jmv.26097
   PMid:32516845
6. Poudel A, Poudel Y, Adhikari A, Aryal BB, Dangol D,

Bajracharya T, *et al*. D-dimer as a biomarker for assessment of COVID-19 prognosis: D-dimer levels on admission and its role in predicting disease outcome in hospitalized patients with COVID-19. PLoS One. 2021;16(8):e0256744. https://doi.org/10.1371/journal.pone.0256744
PMid:34437642

7. Simadibrata DM, Calvin J, Wijaya AD, Ibrahim NA. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: A meta-analysis. Am J Emerg Med. 2021;42:60-9. https://doi.org/10.1016/j.ajem.2021.01.006
PMid:33453617
8. Indonesian Society of Respirology, Indonesian Hearth Association, Indonesian Society of Internal Medicine, Indonesian Society of Anesthesiology and Intensive Therapy, Indonesian Pediatric Society. Pedoman Tatalaksana COVID-19. 2nd ed. Jakarta: Indonesian Society of Respirology; 2020.
9. Kerboua KE. NLR: A cost-effective nomogram to guide therapeutic interventions in COVID-19. Immunol Invest. 2021;50(1):92-100. https://doi.org/10.1080/08820139.2020.1773850
PMid:32482134
10. Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, *et al*. Clinical value of immune-inflammatory parameters to assess the severity of Coronavirus disease 2019. Int J Infect Dis. 2020;95:332-9. https://doi.org/10.1016/j.ijid.2020.04.041
PMid:32334118
11. Jafarzadeh A, Jafarzadeh S, Nozari P, Mokhtari P, Nemati M. Lymphopenia an important immunological abnormality in patients with COVID-19: Possible mechanisms. Scand J Immunol. 2021;93(2):e12967. https://doi.org/10.1111/sji.12967
PMid:32875598
12. Zhao Q, Meng M, Kumar R, Wu Y, Huang J, Deng Y, *et al*. Lymphopenia is associated with severe Coronavirus disease 2019 (COVID-19) infections: A systematic review and meta-analysis. Int J Infect Dis. 2020;96:131-5. https://doi.org/10.1016/j.ijid.2020.04.086
PMid:32376308
13. Zheng Y, Xu H, Yang M, Zeng Y, Chen H, Liu R, *et al*. Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu. J Clin Virol. 2020;127:104366. https://doi.org/10.1016/j.jcv.2020.104366
PMid:32302954
14. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018;9:754. https://doi.org/10.3389/fimmu.2018.00754
PMid:29706967
15. Smilowitz NR, Kunichoff D, Garshick M, Shah B, Pillinger M, Hochman JS, *et al*. C-reactive protein and clinical outcomes in patients with COVID-19. Eur Heart J. 2021;42(23):2270-9. https://doi.org/10.1093/eurheartj/ehaa1103
PMid:33448289
16. Mousavi-Nasab SD, Mardani R, Azadani HN, Zali F, Vasmehjani AA, Sabeti S, *et al*. Neutrophil to lymphocyte ratio and C-reactive protein level as prognostic markers in mild versus severe COVID-19 patients. Gastroenterol Hepatol Bed Bench. 2020;13(4):361-6.
PMid:33244379
17. Sukrisman L, Sinto R, Priantono D. Hematologic profiles and correlation between absolute lymphocyte count and neutrophil/lymphocyte ratio with markers of inflammation of COVID-19 in an Indonesian national referral hospital. Int J Gen Med. 2021;14:6919-24. https://doi.org/10.2147/IJGM.S337440
PMid:34703296
18. Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H, *et al*. Diagnostic value of D-dimer in COVID-19: A meta-analysis and meta-regression. Clin Appl Thromb Hemost. 2021;27:10760296211010976. https://doi.org/10.1177/10760296211010976
PMid:33926262
19. Rostami M, Mansouritorghabeh H. D-dimer level in COVID-19 infection: A systematic review. Expert Rev Hematol. 2020;13(11):1265-75. https://doi.org/10.1080/17474086.2020.1831383
PMid:32997543
20. Poudel A, Poudel Y, Adhikari A, Aryal BB, Dangol D, Bajracharya T, *et al*. D-dimer as a biomarker for assessment of COVID-19 prognosis: D-dimer levels on admission and its role in predicting disease outcome in hospitalized patients with COVID-19. PLoS One. 202;16(8):e0256744. https://doi.org/10.1371/journal.pone.0256744
PMid:34437642
21. Isbaniah F, Juliani T, Damayanti T, Yenita D, Yunus F, Antariksa B, *et al*. The role neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and D-dimer in predicting the outcome of confirmed COVID-19 patients. J Respir Indones. 2021;41(4):236-44.
22. Man MA, Rajnoveanu RM, Motoc NS, Bondor CI, Chis AF, Lesan A, *et al*. Neutrophil-to-lymphocyte ratio, platelets-to-lymphocyte ratio, and eosinophils correlation with high-resolution computer tomography severity score in COVID-19 patients. PLoS One. 2021;16(6):e0252599. https://doi.org/10.1371/journal.pone.0252599
PMid:34181675