High Neutrophil to Lymphocyte Ratio, C-Reactive Protein, Procalcitonin and D-dimer as Risk Factors for Severe COVID-19

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Abstract

Background: COVID-19 is caused by the SARS-CoV2 virus which is a new type of virus that causes global morbidity and mortality. Several studies are needed to determine biomarkers to identify the severity of COVID-19. **Objective:** to analyze the correlation between Neutrophil to lymphocyte rate (NLR), C-Reactive Protein (CRP), D-dimer, dan Procalcitonin (PCT) on the severity of COVID-19. **Method:** A retrospective case-control study was done in our institution using data from June 2020 to March 2021. Data collected were serum NLR, CRP, d-dimer and PCT. Data were analysed using SPSS. Participants were divided into 2 groups, severe COVID-19 as case and mild to moderate as control group. The data collection was carried out in June 2020 – March 2021, included NLR, CRP, D-dimer, and PCT in patient serum. Data analysis using Chi-Square test and logistic regression with $p < 0.05$. **Result:** The NLR value of participants in case group is 11.4 ± 9.7 and in control group is 8.2 ± 8.5 (95% CI $1.081 – 4.641$; $p = 0.023$). The PCT value of participants in case group was 10.3 ± 75.4 and in control group was 6.9 ± 41.4 (95% CI $1.495 – 6.908$; $p < 0.001$). CRP values in case group (123.7 ± 108.9) were higher than control group (61.3 ± 60.8; 95% CI $1.181 – 5.063$; $p < 0.001$). Meanwhile, the value of D-dimer participants in case group was 3.5 ± 3.7 and control group was 2.7 ± 4.6 (95% CI $0.604 – 2.958$; $p = 0.473$). **Conclusion:** increased values of NLR, CRP, and D-dimer are risk factors for severe COVID-19.

Keyword: Neutrophil to Lymphocyte Ratio, C-Reactive Protein, D-dimer, Procalcitonin, the severity of COVID-19

Introduction

Acute respiratory infections caused by viruses are one of the leading causes of morbidity and mortality globally. The World Health Organization (WHO), China Country Office reported a case of pneumonia of unknown etiology in the city of Wuhan, Hubei province, China called COVID-19[1]. Clinical symptoms experienced by patients vary widely from asymptomatic to signs of respiratory failure. The severity of the COVID-19 disease is an important concern in handling the disease. Patients with severe COVID-19 have symptoms of respiratory failure and require mechanical ventilation and intensive care unit (ICU) care with a mortality rate of 50-60%. This is the basis for the importance of evaluating risk factors that

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affect the severity of COVID-19 patients[2].

The development of biomarker examinations for lung disease in the world has helped a lot in determining the severity of the disease. Research conducted in China on risk factors that influence the severity of COVID-19 shows that D-dimer examination is a risk factor for the severity of COVID-19 disease and patient prognosis[3]. In addition, inflammatory markers such as C-Reactive Protein (CRP), Procalcitonin (PCT), Neutrophil to Lymphocyte Ratio (NLR), can affect the severity of COVID-19. NLR is an inflammatory biomarker that can be used as an indicator of the systemic inflammatory process. These biomarkers were determined by dividing the absolute neutrophil and absolute lymphocyte counts. Elevated NLR may serve as a risk and prognostic factor for the severity of COVID-19[4, 5]. Meanwhile, CRP is a protein produced by the liver in the context of inflammation in the body. Patients with CRP >41.8 mg/L can lead to a severe inflammatory process and worsen the degree of COVID-19[6].

PCT is an examination related to bacterial infection where this examination can show the presence of bacterial infection and severe viral infection. So, this examination can play a role in determining the severity of COVID-19. D-dimer is a blood coagulation marker in COVID-19 patients that is used to monitor co-occurring diseases that are the cause of the increasing mortality rate of COVID-19. D-dimer levels above 1.0 g/mL are associated with patient mortality[3, 6, 7].

Several studies conducted in various countries showed an association between PCT, CRP, NLR, and D-dimer in determining mortality in COVID-19 patients. This is the basis for researchers to examine the examination of biomarkers NLR, CRP, D-dimer, and PCT as risk factors for the severity of COVID-19 disease. The purpose of this study is to analyse the inflammatory and coagulation processes that occur and determine the risk factors for the severity of COVID-19.

Method

Participant

Participants in this study were patients who had SARS-CoV2 virus infection confirmed by an RT-PCR swab in the oronasopharynx. Participant inclusion criteria include COVID-19 patients, aged >18 years, and COVID-19 patients hospitalized until they were allowed to go home or die. Participant with incomplete data was excluded from this study

Design Study

A restrospective case-control study was done in Sanglah General Hospital. This research was conducted in the period June 2020 to March 2021. The number of participants in this study was 180 participants and divided into 2 groups (case group = COVID-19 with severe degrees and control group = COVID-19 with moderate-mild degrees). Data collected were age, length of stay, the severity of COVID-19 at hospital admission, PCT levels, CRP levels, D-dimer levels, and NLR ratio. NLR ratio is the absolute number of neutrophils compared to the absolute number of lymphocytes obtained from the patient's complete blood count. Levels of CRP was obtained from the patient's blood with standard CRP test. Procalcitonin is a precursor level of the hormone calcitonin D which is physiologically synthesized by the C cells of the thyroid gland. D-dimer epitope levels in the D-dimer fragment using the Enzyme-Linked Immunosorbent Assay (ELISA) examination method from the patient's blood.

Statistical Analysis

The data in this study were first tested for normality using the Kolmogorov Smirnov test. This analysis was assisted using IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY,

USA). The statistical test used in this study used the Chi-Square test and logistic regression with $p < 0.05$.

Result

Characteristic of Participant

Most of the participants were male with a total of 117 people and 65% of the samples (p = 0.160). The mean age of the participants was 54.41 ± 14.17 years. Some participants recovered and returned alive, of which 63% were severe participants and 86% were non-severe participants ($p = 0.002$). Some participants had comorbid diseases including diabetes mellitus (18% vs 13%; $p = 0.411$), heart defects (21% vs 6%; $p = 0.022$), and kidney disorders (17% vs 9%; $p = 0.037$; table 1).

NLR, CRP, D-dimer, and PCT as risk factors for the severity of COVID-19

The NLR value of participants in case group was 11.4 ± 9.7 and in control group it was 8.2 ± 8.5 (95% CI 1.081 – 4.641; $p = 0.023$). The PCT value of participants in case group was 10.3 ± 75.4 and in control group was 6.9 ± 41.4 (95% CI 1.495 – 6.908; $p < 0.001$). CRP values in case group (123.7 ± 108.9) were higher than control group (61.3 ± 60.8; 95% CI 1.181 – 5.063; $p < 0.001$). Meanwhile, the D-dimer value of participants in case group was 3.5 ± 3.7 and control group was 2.7 ± 4.6 (95% CI 0.604 – 2.958; $p = 0.473$; table 2).

Combination of NLR, CRP, D-dimer, and PCT as a Risk Factor for Severity of COVID-19

Based on the results of multivariable analysis, it was found that 4 variables that have a significant impact on the severity of COVID-19 are NLR, CRP, D-dimer, and heart defects. Then, multiple logistic regression analysis was performed to obtain the adjusted odds ratio (table 3). Based on the results of the chi-square analysis, the sensitivity of the scoring system was 78.9% with a specificity of 60%. These results suggested that the combination of NLR with CRP, or with D-dimer and cardiac abnormalities may be a risk factor for the severe COVID-19.

Table 1. Characteristics of participants

Variable	COVID-19 Degree		p
	Severe (%) n = 90	Mild to moderate (%) n = 90	
Gender			
Man	63 (70)	54 (60)	0.160
Woman	27 (30)	36 (40)	
Heart defects			
Yes	19 (21)	6 (6)	0.022*
Not	71 (79)	84 (94)	
Diabetes mellitus			
Yes	16 (18)	12 (13)	0.411
Not	74 (82)	78 (87)	
Patient Dies			
Die	33 (37)	15 (17)	0.002*
Come back alive	57 (63)	75 (83)	
Kidney disorders			
Yes	15 (17)	8 (9)	0.037*
Not	75 (83)	82 (91)	

Table 2. Analysis of risk factors for NLR, CRP, D-dimer and Procalcitonin on the degree of COVID-19

variable	COVID-19 Degree		OR	95% CI	p
	Severe	Mild to moderate			
NLR	11.4±9.7	8.2±8.5	2.240	1.081 – 4,641	0.023*
Procalcitonin	10.3±75.4	6.9 ± 41.4	3.214	1.495 – 6.908	< 0.001**
CRP	123.7 ± 108.9	61.3 ± 60.8	2,550	1.181 – 5.063	< 0.001**
D-Dimer	3.5±3.7	2.7±4.6	1.337	0.604 – 2.958	0.473

Table 3. Analysis of multivariate risk factors for severe COVID-19

Variable	OR	95% CI	p
NLR	2.360	1.176 – 4.738	0.016 *
CRP	3,432	1.665 – 7.073	0.001 *
D-dimer	2.804	1,404 – 5,060	0.003 *
Heart defects	5.325	1,767 – 16,046	0.003 *

Discussion

Neutrophyl to Lymphocite Ratio is a marker of inflammatory processes that occur in the body. Several studies stated that NLR levels were significantly different between the severe and mild to moderate COVID-19 groups[6]. Other studies have also stated high levels of NLR as a prognostic marker that affects the worsening of COVID-19[8]. Previous studies have also shown a positive correlation between NLR and severe COVID-19[9]. The NLR levels were found to be elevated in viral infections such as influenza, coronavirus, and rhinovirus. The NLR levels are elevated in patients with severe SARS-CoV2 virus infection. The higher the NLR level, the more severe the inflammation that occurs in the patient[6, 8, 9]. The results of this studies were connected with previous studies which stated that there was a significant difference between severe and non-severe COVID-19 groups[6]. C-Reactive protein is one of the markers of inflammation that increases when there is exposure to microbes or irritating substances. High CRP levels (> 20.44) in plasma are associated with the severity of COVID-19[10]. High CRP levels are associated with severity, Deep Vein Trombosis (DVT), renal impairment, and death in COVID-19[11]. The CRP levels as a marker of the inflammatory response is associated with the severity of COVID-19[12]. High CRP levels are a marker of an acute inflammation that occurs in the body. Level of CRP were found to be elevated in viral infections such as influenza, coronavirus, and rhinovirus. CRP levels are elevated in patients with severe SARS-CoV2 virus infection. The increase in CRP levels, the more severe the inflammation that occurs in the patient[6, 10]. This result is different with the results of previous studies

where PCT levels between severe and non-severe COVID-19 were significantly different. This is because PCT is increased in severe viral infections. PCT is an increased investigation specific for bacterial infections. However, several studies have shown an increase in PCT levels in patients with severe viral infections[6]. A meta-analysis study stated that PCT levels were not associated with the need for intensive care, so PCT levels were not yet a risk factor for severe COVID-19. Thus, it is necessary to conduct further research involving samples in >2 different treatment sites[13].

We found that there are significant differences in D-dimer, which also found in a previous study where D-dimer values increased in COVID-19 patients[14]. D-dimer levels in severe COVID-19 patients were significantly different from non-severe COVID-19. This is due to an increase in thrombosis due to the high inflammation caused by SARS-CoV2 virus infection in the lungs[15]. The likelihood of severe COVID-19 is associated with D-dimer levels >0.5 g/ml[14, 15]. The level of D-dimer is a marker of fibrin formation in the body of COVID-19 patients in the early phase. This could be because infection with the SARS-CoV2 virus can cause an increase in fibrin formation in the patient's body so that D-dimer levels will increase according to the severity of COVID-19 disease. In addition, comorbid illnesses that COVID-19 patients have can also increase D-dimer levels. D-dimer levels may increase due to other illnesses suffered by COVID-19 patients[3]. D-dimer is a risk factor for the severity of COVID-19[9].

We search several studies to determine the combination of biomarkers to predict the severe COVID-19. The NLR has high sensitivity as a marker of severe COVID-19[16]. High levels of NLR and CRP have a high hazard ratio to the severity of COVID-19[6]. The combination of 6 variables (serum amyloid A, interleukin-6, lactate dehydrogenase,

neutrophil-to-lymphocyte ratio, D-dimer, cardiac troponin, renal biomarkers, lymphocytes, and platelet count as a marker of COVID-19 severity[17]. Ponti *et al* also suggested that elevations of the biomarkers NLR, CRP, erythrocyte sedimentation rate (ESR), PCT, interleukin (IL)-6, D-dimer, troponin, creatine kinase (CK), and aspartate aminotransferase (AST) were associated with the severity of COVID-19[18]. Further research is needed on the sensitivity and specificity of this scoring using several samples adjusted for diagnostic tests.

Conclusion

High NLR ratio, D-dimer levels, and high CRP levels are risk factors for severe COVID-19. Meanwhile, high levels of procalcitonin are not a risk factor for the severe COVID-19. The combination of CRP, NLR, and D-Dimer as risk factors for the severe COVID-19.

Conflict of Interest: The authors declare that they have no conflict of interest.

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