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ARTIKEL PENELITIAN

The Enhancement of COVID-19 Severity on Coagulation Markers: a Cross-sectional Study in West Sumatra, Indonesia

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Abstrak

Tujuan: Penelitian ini bertujuan untuk mengetahui gambaran penanda koagulasi dan implikasinya pada pasien COVID-19 berdasarkan luaran klinis berupa peningkatan keparahan dan kematian. **Metode:** Subyek dikumpulkan dari pasien positif COVID-19 RT-PCR dengan catatan medis termasuk data demografi, dan hasil laboratorium seperti hemoglobin, hematokrit, leukosit, trombosit, penanda koagulasi (D-dimer, PT, dan APTT), dan hasil pasien pasca perawatan. Analisis statistik dilakukan dengan menggunakan uji chi-square dan uji Mann-Whitney yang secara statistik dianggap perbedaan tingkat signifikan antar variabel. **Hasil:** Pasien COVID-19 diklasifikasikan menjadi kelompok ringan sebanyak 27 subjek (44,2%) dan kelompok sedang-berat sebanyak 34 subjek (55,8%). Kadar leukosit, D-dimer, dan PT berbeda secara signifikan antara kelompok ringan dan sedang-berat. Kami menemukan berdasarkan uji Mann-Whitney bahwa peningkatan pada kelompok ringan-berat dan tidak selamat cenderung diikuti oleh perubahan penanda koagulasi berupa peningkatan D-dimer dan pemanjangan PT. Sedangkan faktor hematologi menunjukkan bahwa pada pasien COVID-19, efek peningkatan leukosit dan penurunan hematokrit jauh lebih besar kemungkinannya untuk diikuti dengan peningkatan gejala parah dan kematian. **Kesimpulan:** Penelitian ini menunjukkan adanya hubungan yang bermakna antara kelainan penanda koagulasi dengan derajat keparahan dan kematian. Semua penanda koagulasi mempunyai efek gejala pada pasien Covid-19, peningkatan D-dimer dan pemanjangan waktu protrombin pada pasien COVID-19 dewasa cenderung diikuti dengan peningkatan gejala parah dan kematian.

Kata kunci: COVID-19, tingkat keparahan, penanda koagulasi, D-dimer, tromboplastin

Abstract

Objective: *This study aimed to determine the description of the coagulation marker and how it implicates COVID-19 patients based on clinical outcomes in the form of enhancement severity and death.* ***Methods:*** *The subjects were collected from patients with positive COVID-19 RT-PCR with medical records including demographic data, and laboratory results such as hemoglobin, hematocrit, leukocytes, platelets, coagulation markers (D-dimer, PT, and APTT), and patients outcomes post-treatment. Statistical analysis is performed by using chi-square and Mann-Whitney tests were considered statistically difference level of significant between variables.* ***Results:*** *The COVID-19 patients is classified into mild group with 27 subjects (44.2%) and moderate-severe group 34 subjects (55.8%). Leukocytes, D-dimer, and PT levels significantly differed between mild and moderate-severe groups. We found based on Mann-Whitney test that the enhancement of the mild-severe and the non-*

survivor group tended to be followed by changes in coagulation markers in the form of increased D-dimer and PT prolongation. While the hematological factors indicate that in COVID-19 patients, the effect of the increased leukocyte and decreased hematocrit is substantially more likely to be followed by more enhanced severe symptoms and death. ***Conclusion:*** *This study indicated a significant relationship between the abnormalities of coagulation markers with severity and death. All coagulation markers had a symptomatic effect on Covid-19 patients, increased D-dimer and prolonged prothrombin time in adult COVID-19 patients tend to be followed by the enhancement of severe symptoms and death.*

Keywords: COVID-19, severity, coagulation markers, D-dimer, thromboplastin

INTRODUCTION

In the first three years of the global pandemic, coronavirus disease 2019 (COVID-19) impacted various sectors, including health.[1] From the first case until February 2023, COVID-19 has caused 161.000 mortality in Indonesia. Clinically, COVID-19 can cause various severities ranging from asymptomatic, mild, moderate, and severe to critical.[2-4] Severe patients can develop to a critical phase characterized by worsening of the respiratory system, such as acute respiratory syndrome disease (ARDS).[5]

Coagulation disorders are the main complication that most often occurs in severe COVID-19 patients.[4,6] Hypercoagulation or hypercoagulopathy was a factor that increased the development of acute respiratory distress syndrome (ARDS). Hypercoagulopathy was defined as excessive blood clotting or clotting. Hypercoagulopathy increases the tendency for a person to experience pathological micro and/or macro arterial or venous thrombosis, such as venous thromboembolism, stroke, thrombotic microangiopathy, and downregulation of protein C signaling.[7-8] Several pathomechanisms have been linked to coagulation disorders in COVID-19.[9-11] Impaired vascular endothelial function and damage, hyperinflammation, and changes in plasma pro-coagulant and fibrinolytic factor activity are the basis of coagulation disorders in COVID-19.

Acute COVID-19 infection is accompanied by a vast list of symptoms that are caused by the virus, the host, and downstream effects. The result of these mechanisms is that the coagulation abnormality continues to be persistent. The effects of coagulation disorder were also discovered in the long COVID-19 patients,[12] where the abnormality of coagulation is associated with infection with concurrent cardiovascular disorders needing anti-platelet and anticoagulant.[13-15]

In Indonesia, clinical monitoring of coagulation disorders is widespread and routine using D-Dimer, prothrombin time (PT), and activated partial thromboplastin time (APTT). This study aims to determine the coagulation implication on COVID-19 patients.

METHODS

Research Subject

This study was a single-center cross-sectional observational study. The subjects were 61 patients with positive COVID-19 RT-PCR and above 18 years old who were treated at Dr. M. Djamil Hospital in Padang from November 2021 to November 2022. We excluded subjects with incomplete data and subjects with conditions of pregnancy, HIV, and liver cirrhosis diseases.

Data Collections

The study data were collected from medical records, including demographic data, age, gender, comorbid diseases, laboratory results such as hemoglobin, hematocrit, leukocytes, platelets, coagulation markers (D-dimer, PT, and APTT), and patients outcomes post-treatment. The subjects were divided into mild and moderate-severe groups based on the severity of COVID-19 symptoms. A mild group had mild clinical symptoms such as fever, cough, fatigue, anorexia, myalgia, sore throat, headache, nasal congestion, diarrhea, nausea, vomiting, anemia, and peripheral oxygen saturation (SpO2)≥96%. The moderate-severe group was subjects who had moderate-severe

COVID-19 clinical symptoms such as pneumonia, shortness of breath, ARDS, and severe respiratory distress with SpO2<96%.

Statistical Analyses

The measured data were presented as mean ± standard deviation if normally distributed or as median (min-max) if abnormally distributed. The chi-square and Mann-Whitney tests were used to analyze the data, and p<0.05 were considered statistically significant.

RESULTS AND DISCUSSION

A total of 61 patients were included in this study. The mild group was 27 subjects (44.2%), and the moderate-severe group was 34 subjects (55.8%). The moderate-severe group had higher median age than the mild group. The gender shows that 17 (66.3%) females dominated the mild group, and 10 (33.7%) males dominated the moderate-severe group. The moderate-severe group tended to have more comorbidities. Hypertension was the most common comorbidity in 17 (50%) of subjects.

Leukocytes, D-dimer, and PT levels significantly differed between mild and moderate-severe groups. The details are shown in Table 1 and Figure 1. In the moderate-severe group, leukocytosis was more likely to occur (22 of 34 subjects), but in the mild group, only 1 of 27 subjects. D-dimer levels tended to increase in moderate-severe patients. There were 6 out of 27 mild subjects with D-dimer levels above normal. On the other hand, 31 of the 34 moderate-severe groups had D-dimer levels above normal, and two had levels >10.000. PT prolongation tended to occur more often in moderate-severe patients than in the mild group, with p = <0,001.

Table 1. Baseline characteristics and laboratory workup according to the severity.

Characteristics	Mild (n=27)	Moderate-severe (n=34)	p-value
Age	40±15.9	57±14.1	**<0.001**
Male	10 (33.7)	20 (66.7)	0.559
Comorbidities			
Hypertension	2 (7.4)	17 (50)	**0.006**
Diabetes	-	10 (29.4)	**0.002**
Ischemic heart disease	-	1 (2.9)	1.000
Renal failure	-	13 (38.2)	**<0.001**
Hematology laboratory work-up			
Haemoglobin (g/dl)	13.1±1.7	12.4±2.6	0.201
Hematocrit (%)	41.8±6.2	36.2±7.9	**0.048**
Leucocytes (X10^3/mm^3)	5.95 (3.4-15)	11.9 (4.4-27.7)	**0.003**
Platelets (X10^3/mm^3)	187 (135-561)	222±114	0.283
Coagulation marker			
D-dimer (ng/ml)	290 (100-1160)	2907.8 (289-10000)	**<0.001**
Prothrombin time (s)	9.6 (8.1-66.5)	11.4 (9.1-19)	**<0.001**
Activated partial thromboplastin time (s)	27.4 (20.1-73.8)	28.4 (20.3-56.5)	0.412

In this study, 21 (32%) patients were non-survivors and tended to have a higher age than survivors. The levels of haemoglobin, hematocrit, and platelets were not statistically significantly different between the survivor and non-survivor groups, while the levels of leukocytes, D-dimer, and PT were found to be different between the survivor and non-survivor patient groups. Leukocytosis, increased D-dimer, and prolonged PT tended to occur in the non-survivor patient group. The details are shown in Table 2 and Figure 2.

Table 2. Baseline characteristics and laboratory workup according to the death.

Characteristics	Survivor (n=40)	Non-survivor (n=21)	p-value
Age	46 (18-79)	61.3±11.4	**<0.001**
Male	17 (42.5)	9 (42.9)	1.000
Comorbidities			
Hypertension	8 (20)	11 (52.4)	**0.021**
Diabetes	4 (7.5)	7 (33.3)	**0.024**
Ischemic heart disease	-	1 (1.6)	0.344
Renal failure	2 (5)	11 (52.4)	**<0.001**
Hematology laboratory work-up			
Haemoglobin (g/dl)	12.7±1.8	11.9±3.1	0.232
Hematocrit (%)	39.8±6.7	36.3±9	0.093
Leucocytes (X10^3/mm^3)	9.69 (3.4-27.75)	10.83±3.22	**0.002**
Platelets (X10^3/mm^3)	191.5 (38-561)	199±81.8	0.687
Coagulation marker			
D-dimer (ng/ml)	393.5 (100-7443)	2633 (289-10000)	**<0.001**
Prothrombin time (s)	10.15 (8.1-66.5)	11.5 (9.9-19)	**<0.001**
Activated partial thromboplastin time (s)	28 (20.1-73.8)	28.4 (20.3-56.5)	0.395

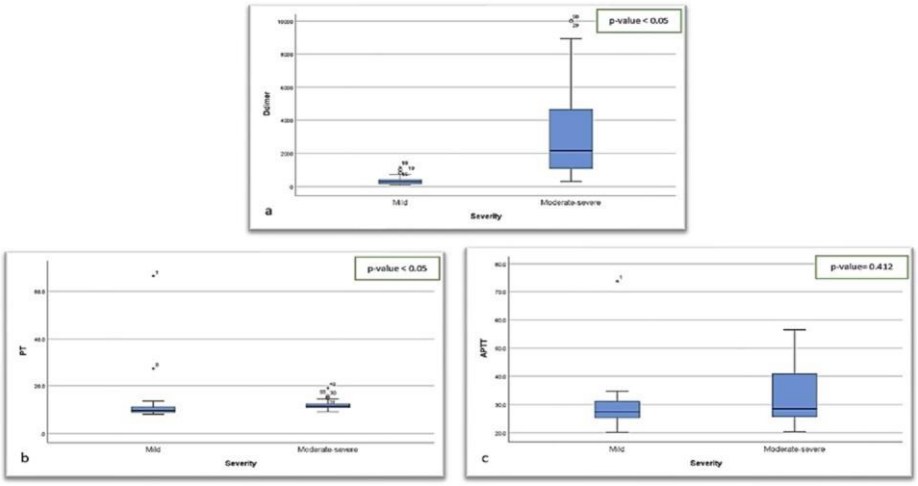

Figure 1. Mean of D-dimer, prothrombin time and activated partial thromboplastin time of mild and moderate-severe COVID-19 patients. (a) D-dimer (b) Prothrombin time; and (c) Activated partial thromboplastin time.

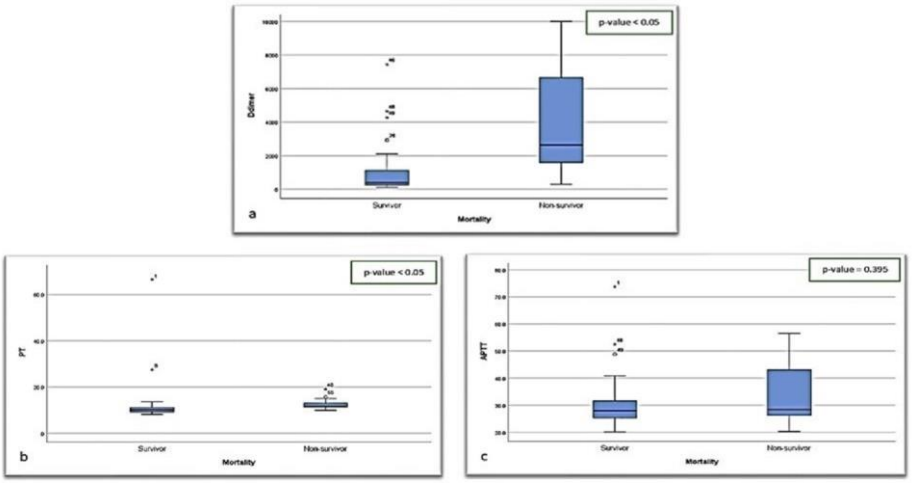

Figure 2. Mean of D-dimer, prothrombin time and activated partial thromboplastin time of survivor and non-survivor COVID-19 patients. (a) D-dimer (b) Prothrombin time; and (c) Activated partial thromboplastin time.

Table 3. Test statistics of Mann-Whitney for hematology variables

	Haemoglobin	Hematocrit	Leucocytes	Platelets
Mann-Whitney U	339.500	276.500	79.500	385.000
Wilcoxon W	934.500	871.500	457.500	763.000
Z	-1.736	-2.651	-5.511	-1.075
Asymp. Sig. (2-tailed)[a]	0.083	0.008	0.000	0.283

a. Grouping Variable: Status of patients with mild and moderate-severe

Table 4. Test statistics of Mann-Whitney for coagulation markers

	D-dimer	Prothrombin Time (PT)	Activated Partial Thromboplastin Time (APTT)
Mann-Whitney U	43.000	200.000	402.500
Wilcoxon W	421.000	578.000	780.500
Z	-6.041	-3.764	-0.820
Asymp. Sig. (2-tailed)[a]	0.000	0.000	0.412

a. Grouping Variable: Status of patients with mild and moderate-severe

Based on inferential analysis studies using the Mann-Whitney test, they are significant differences between the condition mild and moderate-severe caused by the leukocyte and hematocrit, while they are no effects for haemoglobin and platelets as shown in Table 3. Furthermore, D-dimer and prothrombin as coagulation markers that has a significant effect in determining whether a patient's condition is mild or moderate-severe as shown in Table 4. This provides information that the coagulation marker D-dimer and prothrombin can be used as alarm symptoms in treating Covid-19 patients as well as hematology variables of hematocrit and leucocytes.

Our study found that abnormalities of coagulation markers, such as increased D-dimer and prolonged PT, occurred in the moderate-severe group. The results of this study slightly contradicted a study in China with 43 subjects, which found that severe COVID-19 patients tend to have shortened PT and APTT values compared to mild patients, but not statistically significant.[6] A descriptive study on 99 COVID-19 patients in Wuhan, China, found that 16 and 30 patients had results below normal values for APTT and PT, respectively, and 36 patients had D-dimer levels that exceeded normal limits.[4] A study in Saudi Arabia on 284 mild and severe COVID-19 patients revealed that blood coagulation marker abnormalities in increased D-dimer and APTT prolongation occurred in severe COVID-19 patients.[9] A meta-analysis study with 13 articles with 1341 patients revealed that coagulation marker dysfunction was related to the severity of patients with COVID-19, where thrombocytopenia, increased D-dimer, and fibrinogen at admission can be risk factors for increased disease progressivity. PT and APTT tend to be prolonged in severe patients but are not statistically different.[10]

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are clinical coagulation tests used to determine coagulation pathway defects. PT was assessed for extrinsic and shared pathway function, while APTT was assessed for intrinsic pathway function. The imbalance between coagulation and inflammation in SARS-CoV-2 infection results in a hypercoagulable state. An increase in D-dimer and fibrinogen characterizes hyper-coagulation.

Several mechanisms underlying the coagulation dysfunction in severe COVID-19 patients have been hypothesized. Granulate-macrophage colony-stimulating factor (GM-CSF) and interleukin-6 (IL-6) are two examples of pro-inflammatory cytokines that can be secreted by pathogenic Th-1 cells when the SARS-CoV-2 virus is present. Monocytes are further activated by GM-CSF, which causes them to release a lot of IL-6, tumor necrosis factor (TNF), and other cytokines. In-vitro, most pro-inflammatory cytokines have been shown to have the ability to activate coagulation systems such as fibrinogen and factor VII.[16] Tissue factor (TF), fibrinogen, and factor VIII expression are all inducted by IL-6 in inflammatory tissues. Through the action of C5a, which activates tissue factor mannan-binding lectin serine protease (MASP)-1, which cleaves fibrinogen and factor XIII resulting in coagulation, intrinsic and extrinsic coagulation pathways interacting with complement factors induce pro-coagulation. The normal or slightly extended PT and normal APTT in the majority of patients with COVID-19 are most likely explained by high levels of factor VIII and fibrinogen.[11] One study showed that elevated D-dimer in critically ill patients was associated with an increased risk of thrombotic factors. Patients' rates of thrombosis and bleeding may be related to their critical stage.[17]

Most deaths from COVID-19 occur in severe and critical patient groups. Various factors associated with mortality in COVID-19 patients have been widely recognized, such as old age, comorbidities, and COVID-19 vaccination status.[18] ARDS was the most important cause of COVID-19 mortality. Patients with complications of coagulation disorders have a higher risk of death. Our study found that the non-survivor group tends to be accompanied by an increased D-dimer and prolonged PT at the beginning of hospitalization. A study in China with

449 COVID-19 patients also found that prolonged PT and D-dimer increases tended to occur in the non-survivor patient group.[19]

This study has several limitations. First, the sample of this study was taken retrospectively through medical records with only 61 selected patients based on certain criteria of research. Second, there is no serial coagulation marker analysis performance due to limited data information. Even within this limitation, the results of this research still provide a strong analytical challenge to the study of enhancement of COVID-19 severity on coagulation markers as a cross-sectional analysis.

CONCLUSION

This study concludes a significant relationship between coagulation markers and COVID-19 patients' severity. Cytokines storm in severe COVID-19 activated coagulation cascade caused increased D-dimer and prolonged prothrombin time that induced ARDS tend to worsen severity and death. Increased D-dimer and prolonged prothrombin time in COVID-19 patients tend to be followed by more severe symptoms and death. They also occur for hematology variables of leukocyte and hematocrit. Increased leukocyte in COVID-19 patients tends to be followed by more severe symptoms and death. Contrary, decreased hematocrit in COVID-19 patients tends to be followed by more severe symptoms and death.

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ETHICAL CONSIDERATIONS

This research protects and maintains the confidentiality of patients data and obtained ethical clearance with research approval number 454/KEPK/2021.

CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

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