RESEARCH ARTICLE

REVISED The improvement of inflammatory markers and disease progression among moderate, severe, and critical COVID-19 patients: a cross-sectional study from two second referral hospitals in Surabaya, Indonesia [version 2; peer review: 1 approved with reservations]

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Abstract

Background: To date, coronavirus diseases 2019 (COVID-19) has no definitive treatment. Thrombosis and hypercoagulation may occur in the advanced stage. Further study on how to use anticoagulants is still required to promote the best prognosis.

Methods: A cross-sectional study of 110 moderate, 140 severe, and 81 critical patients receiving unfractionated heparin (UFH), low-molecular-weight heparin (LMWH), and fondaparinux was conducted. Data were collected from March 15th to August 31st 2020 at Universitas Airlangga and Husada Utama Hospital. A comparative study of white blood cell (WBC), neutrophils, lymphocytes, neutrophil-lymphocyte

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ratio (NLR), c-reactive protein (CRP), procalcitonin (PCT), D-dimer, all-cause mortality rate, length of stay, and days of death among three severities of COVID-19 was done. Univariate and multivariate analysis were used to determine the correlation between inflammatory state after anti-coagulant with patients' mortality.

Results: Two deaths occurred in moderate cases, 36 deaths in severe cases, and 70 deaths in critical cases on ventilators. On day 13, moderate and severe groups showed decreased WBC, neutrophils, NLR, CRP, and D-dimer ($p < 0.05$). NLR, CRP, and D-dimer ($p < 0.05$) in critically ill and ventilated patients decreased. Day-13 evaluation revealed 32.73% decrease of inflammatory markers in moderate group; 32.86% in severe patients; and 16.05% in critically ill, ventilated patients. A significant correlation between day 13 inflammatory status with mortality was seen in moderate and critical cases with a ventilator ($r=0.337$; $p < 0.05$ and $r=0.25$; $p < 0.05$). Inflammatory profile on day 6 (adjusted odds ratio [aOR] = 2.36; $p < 0.05$) and day 13 ([aOR] = 4.15; $p < 0.05$) was associated with patients' mortality.

Conclusions: Anticoagulants in COVID-19 patients lower inflammation markers. Evaluating inflammatory status is essential to predict the mortality. Inflammatory markers on day 13, based on the severity of COVID-19 and comorbidities, were associated with mortality in moderate and critical cases.

Keywords

COVID-19, Anti-coagulant, mortality, length of stay, severity, health

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Any reports and responses or comments on the article can be found at the end of the article.

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There is no change in the main article, just add the new two authors Tri Pudy Asmarawati and Alfian Nur Rosyid.

Any further responses from the reviewers can be found at the end of the article

Introduction

Coronavirus disease 2019 (COVID-19) infection has caused widespread novel COVID-19 pneumonia, causing respiratory problems.¹ The World Health Organization (WHO) declared this as a global pandemic affecting many aspects of life.² A severe degree of COVID-19 pneumonia is defined as a condition in patients complaining of difficulty in breathing, plus one of: respiratory rate >30 times per minute; severe respiratory distress; or oxygen saturation (SpO_2) $<93\%$ in room air or a $\text{PaO}_2/\text{FiO}_2$ ratio (PF Ratio) <300 . In children, it is defined as having a cough or difficulty breathing, plus at least one of: central cyanosis or $\text{SpO}_2 <90\%$; severe respiratory distress (such as snoring, heavy chest wall traction); signs of severe pneumonia, namely inability to suckle/drink; lethargy or decreased consciousness, or seizures. Other signs of pneumonia are chest retractions, rapid breathing $>60\times/\text{minute}$ in children aged <2 months, $>50\times/\text{minute}$ in children aged 2-11 months, $>40\times/\text{minute}$ in children aged 1-5 years, or $>30\times/\text{minute}$ in children aged >5 years.²

Severe SARS-CoV2 infection promotes a syndrome related to prothrombotic conditions, in which blood clots easily. This condition is characterized by several specific abnormal laboratory values, such as mild thrombocytopenia, increased fibrin, degradation of fibrin products, fibrinogen, and D-dimers. Increased D-dimers are strongly correlated with worsened clinical conditions and increased risk of death in COVID-19.^{3,5} Based on reports from several studies, there is a growing incidence of several thromboembolic states in patients with COVID-19 admitted to the intensive care unit (ICU), one of which is pulmonary embolism. COVID-19 patients suspected of experiencing a thromboembolic event should be given anticoagulant therapy when radiological imaging is difficult to perform.³⁻⁵

The pathophysiology of hypercoagulation in COVID-19 patients is still currently being explored. A case series presenting three cases of COVID-19 with antiphospholipid syndrome has recently been reported by The New England Journal of Medicine.⁵ SARS-CoV-2 infection is associated with antiphospholipid antibodies, which predispose to hypercoagulation. The study of Campbell *et al.* reported severe COVID-19 patients with increased levels of lactate dehydrogenase (LDH), D-Dimer, bilirubin, decreased platelets, mild anemia, heart and kidney injury, and diffused thrombotic micro-angiopathy.⁵⁻⁷

Patients with severe COVID-19 experience complications of coagulopathy in the form of disseminated intravascular coagulation (DIC) which might result in death. Severe COVID-19 patients experience respiratory problems and increased virulence, according to the criteria of the Third International Consensus Definitions for Sepsis (Sepsis-3). Severe COVID-19 patients are also at risk of venous thromboembolism (VTE) due to prolonged bed rest. The International Society of Thrombosis and Haemostasis (ISTH) provides a new category to identify the early phase of sepsis-associated DIC which is also called sepsis-induced coagulopathy (SIC). COVID-19 patients who fit the diagnostic criteria for SIC can be also given anticoagulant therapy.⁵⁻¹⁰

Anticoagulant therapy in COVID-19 patients is administered to those who show signs of thrombosis, such as elevated inflammatory factors and D-dimers within 7-14 days, with threefold D-Dimer value. The option would be to use low molecular weight heparin (LMWH) at a dose of 100 IU/kg twice a day, for 3-5 days. The European Society of Cardiology also includes anticoagulants in the COVID-19 therapy algorithm. For patients admitted to the ICU, the parenteral heparin drip protocol must be strictly controlled and the time value of the active prothromblastin time is 60-85 seconds. For non-ICU patients, the subcutaneous dose of enoxaprin is started at 1mg/kg twice daily. After all the completed researches, further study on how to use anticoagulants is still being analysed to promote the best prognosis.^{3,11,12}

Methods**Ethics approval**

Approval was issued by the research ethics board Universitas Airlangga Hospital (No: 179/KEP/2020) on 2nd October 2020. The consent to participate was not applicable since our data were obtained from medical records.

Study design and population

We performed a retrospective cohort study with consecutive sampling among COVID-19 adult patients admitted to two referral hospitals for COVID-19, Universitas Airlangga Hospital (UAH) and Husada Utama Hospital, Surabaya, East Java, Indonesia, from March 15th 2020 to August 31st 2020. The patients included were ones in moderate, severe, or critical condition. We assessed patients referring to WHO guidelines and Indonesian Ministry of Health guidelines, and

tested for SARS-CoV-2 Polymerase Chain Reaction (PCR) through oropharyngeal and nasopharyngeal swabs. Sequential chest x-ray and laboratory inflammatory marker evaluation were performed, then we did three timeframes of assessment. The first evaluation was done at the admission, followed by a second evaluation on the sixth day, and lastly evaluated before discharge or death (mean time 13 days).

Data collection

In October 2020, we collected the data from medical records of those admitted to UAH and Husada Utama hospital between March 15th, 2020 to August 31st, 2020. We had 331 patients with moderate to critically ill COVID-19. Incomplete medical records were excluded. From this selection, we organized patient records into 110 moderate cases, 140 severe cases, and 81 critically ill cases with ventilator (see Figure 1).

Patients were categorized as a moderate case if they had either or both of 1) signs of pneumonia; 2) O₂ sat $\geq 93\%$ free air. Meanwhile, patients were classified into severe cases if there were clinical signs of pneumonia, with one of the following: 1) respiratory rate (RR) >30 times per minutes; 2) severe respiratory distress; 3) oxygen saturation $< 93\%$ free air. They were grouped into critically ill if they suffered from acute respiratory distress syndrome (ARDS), sepsis, and septic shock.²

The criteria of inflammatory markers were: 1) white blood cell (WBC) $>6.16 \times 10^3$ cells/ μL ;¹³ 2) neutrophil-lymphocyte ratio (NLR) >6.5 ;¹⁴ 3) absolute lymphocyte count ALC $< 1.0 \times 10^3$ cells/ μL ;¹⁵ 4) c-reactive protein (CRP) >41.8 mg/L;¹⁶ and 5) Procalcitonin > 0.07 ng/mL.¹⁶

Statistical analysis

We examined the data using SPSS version 24.0 (Chicago, IL, USA). Descriptive analysis incorporated categorical variables reported as number (percentage) and continuous variables as mean (standard deviation). We displayed categorical variables as number (%) and continuous variables as mean (standard deviation) or median (range), depending on whether the data are normally distributed. Means of Chi square or McNemar was used to assess statistical significance for dichotomous variables, while paired t-test or Wilcoxon test were used to examine continuous variables, depending on the data distribution. We compared the mean/median difference of the first to the second evaluation and the second

Figure 1. Selection of patients (CRP: c-reactive protein).

to the third evaluations of the laboratory results, length of stay, days of death, and mortality rate according to severity of COVID-19 using ANOVA or Kruskal Wallis. Bivariate analysis between inflammatory state and mortality was conducted using Spearman. We also determined the relationship between inflammatory state after anti-coagulant with patients' mortality, along with some variables, which were age, gender, disease severity, and comorbidity.

Results

Baseline data

Of 331 patients enrolled, 200 were male and 131 were female. Patients were grouped based on the category of severity of disease and resulted in 110 moderate patients, 140 severe patients, and 81 critically ill patients with ventilator support. The average age of patients with severe COVID-19 was 57.45 ± 13.5 years. In the anticoagulant group, the most frequent comorbid factors found were diabetes mellitus (DM) (52.86%), hypertension (HT) (46.91%), and geriatric age (47.14%) while others had history of heart disease (5.45%), stroke (3.7%), and chronic kidney disease (1.82%). We evaluated inflammatory markers as baseline study data. There were significant differences in laboratory markers between each severity group (p value <0.05). The highest white blood count was $8.53 \pm 5.41 \times 10^3/\text{L}$, neutrophils $83.75 \pm 9.51\%$, lymphocyte $17.1 \pm 9.9\%$, lymphocyte absolute $1.1 \pm 0.57 \times 10^3/\text{L}$, neutrophil lymphocyte ratio (NLR) 8.38 ± 12.5 , the C-reactive protein (CRP) $40.1 \pm 42.43 \text{ mg/L}$ and d-dimer $1.2 \pm 8.53 \text{ mcg/L}$. The outcome analysis showed significant differences in the mean length of stay (p value <0.05) (see Table 1).

Table 1. Characteristics of subjects (DM: diabetes mellitus; HT: hypertension).

	Severity of COVID-19						
	Moderate		Severe		Critically ill with ventilator support		Total
	110		140		81		331
Sex							
Male (n,%)	64	58,18	82	58,57	54	66,67	200
Female (n,%)	46	41,82	58	41,43	27	33,33	131
Age (mean/SD)	53,62	13,67	57,45	13,5	56,11	13,63	167,18
Comorbid							
Geriatric age (age>60 years old) (n,%)	40	36,36	66	47,14	29	35,80	135
DM (n,%)	33	30,00	74	52,86	23	28,40	130
HT (n,%)	40	36,36	54	38,57	38	46,91	132
Heart Disease (n,%)	6	5,45	7	5,00	4	4,94	17
Chronic Kidney Disease (n,%)	2	1,82	2	1,43	1	1,23	5
Stroke (n,%)	1	0,91	4	2,86	3	3,70	8
Laboratory							p Value
Leucocyte ($10^3/\text{uL}$; median, SD)	6,49	3,55	7,166	5,89	8,53	5,41	<0.05*
Neutrophil (%; median, SD)	72,4	15,7	78	21,41	83,75	9,51	<0.05*
Lymphocyte (%; median, SD)	17,1	9,9	12,15	10,42	9,5	7,01	<0.05*
Lymphocyte absolute ($10^3/\text{uL}$, median, SD)	1,1	0,57	0,915	1,31	0,84	0,51	<0.05*
Neutrophil-Lymphocyte Ratio (NLR) (median, SD)	4,18	5,3	6	5,61	8,38	12,5	<0.05*
C-Reactive Protein (mg/L; median, SD)	18,7	39,43	40,1	42,43	34,1	49,28	<0.05*
D-Dimer (mcg/l; median, SD)	0,62	3,2	0,9	7,48	1,2	8,53	<0.05*
Outcome							
Length of Stay (mean, SD)	18,46	9,54	19,54	10,63	14,26	11,5	<0.05*
Discharge (n,%)	108	98,18	104	74,29	11	13,58	
Death (n,%)	2	1,82	36	25,71	70	86,42	

*Kruskal Wallis.

Subgroup analysis

We evaluated and compared inflammatory markers up to three times. To analyze the relationship between the administration of anticoagulants in each severity of COVID-19 cases, we calculated the decrease or increase in these inflammatory markers.

Based on the first and second laboratory evaluations, there were differences in decreasing leukocyte count and D-dimer in severe cases (p 0.03; p 0.026), decreasing neutrophils neutrophil-lymphocyte ratio and CRP in moderate cases, (p 0.004; p 0.028; and p <0.05), and increasing lymphocyte in critically ill cases (p <0.05). In this initial evaluation, there were no differences in inflammatory status between each degree of COVID-19 cases.

In contrast to the initial evaluation, the comparison of the inflammatory status between the second and third examinations found that there were differences in each severity group (moderate (p <0.05); severe (p <0.05) and critically ill (p 0.001)). Inflammation marker examination found decreasing leukocytes in moderate and severe cases (p <0.05; p 0.001), decreasing neutrophils in moderate, severe and critically ill cases (p <0.05; p <0.05; p 0.007), increasing lymphocyte count in moderate cases (p <0.05); increasing absolute lymphocyte in moderate and severe cases (p <0.05; p <0.05), decreasing NLR in moderate, severe and critically ill cases (p <0.05; p <0.05; p <0.05), decreasing CRP in moderate and severe cases (p <0.05; p <0.05), and decreasing d-dimer in all case groups (p <0.05; p <0.05; p <0.05).

With these significant results, we evaluated the inflammatory parameters from the initial to the last examination. In moderate group, there was an improvement in inflammation parameters in all variables (p <0.05). In severe cases, significant improvements in inflammatory parameters were recorded, including decreasing leukocytes, decreasing neutrophils, decreasing lymphocytes, increasing absolute lymphocytes, decreasing NLR, decreasing CRP and d-dimer (p 0.01; p <0.05; p <0.05; p <0.05; p 0.0; p <0.05; p <0.05 p <0.05).

In the critically ill with ventilator support group, significant results were also obtained on each inflammatory marker variable except for decreasing leukocytes (p 0.6). From the clinical aspect, analysis of the use of anticoagulants on day of death found significant differences among groups of severity (p <0.05). Significant correlation of inflammatory status on the thirteenth day with mortality based on patient comorbidities was seen in moderate and critical cases on a ventilator (r =0.337; p <0.05 and r =0.25; p 0.05) (see [Table 2](#) and [Figure 2](#)).

Univariate and multivariate analysis using regression logistic revealed that no decline of inflammatory profile both on day 6 (adjusted odds ratio [aOR] = 2.36; 95% CI: 1.46-3.83, p <0.05) and day 13 (adjusted odds ratio [aOR]=4.15; 95% CI: 2.33-7.42, p <0.05) was related to the mortality event (see [Table 3](#)).

Discussion

COVID-19 has been linked to coagulation disorders which cause various complications. An increase in coagulation parameters, such as D-dimers, is an independent risk factor for death. Patients with D-dimers of more than 1000 ng/mL have a 20 times greater risk of death due to infection.¹⁷ Although the pathogenesis of coagulopathy in COVID-19 cannot be fully explained, the mechanism may resemble septic coagulopathy in bacterial infections. Overabundant pro-inflammatory cytokines increases the level of damage-associated molecular patterns (DAMP). Activation of coagulation factors due to cell and endothelial damage is the most common mechanism of infection. Both the pathogen and DAMP from damaged tissue activate monocytes. The activated monocytes release pro-inflammatory cytokines (IL-1, IL-6, IL-10, TNF- α) and chemokines that animate neutrophils, lymphocytes, platelets, and vascular endothelial cells. The coagulation cascade is commenced by tissue factor and phosphatidylserine on the cells' surface. Healthy endothelial cells retain anti-thrombogenic properties by expressing glycocalyx and binding with anti-thrombin proteins. Damaged endothelial cells change their nature to become more procoagulant due to glycocalyx disorders and loss of anticoagulant proteins.^{7,12,18,19} Markers of hypercoagulation and high levels of inflammatory mediators are consistent with poor outcome in patients having acute respiratory distress syndrome (ARDS) and sepsis. These observations have led to several studies focused on inflammation and coagulation pathways in acute lung injury, ARDS or sepsis.^{17,20,21}

The routine use of anticoagulant regimens in COVID-19 patients requiring hospitalization has been recommended, after several studies found an association between viral inflammation and coagulation disorders.²² Several guidelines for the management of COVID-19 have included anticoagulant regimens regarding COVID-19-related coagulation disorders. Recommendations issued by the Anticoagulation Forum (ACF) and the American College of Chest Physicians (ACCP) selected Low Molecular Weight Heparin (LMWH) over Unfractionated Heparin (UFH) to minimize laboratory evaluation. ACCP also recommends fondaparinux over UFH in patients with a high risk of bleeding, kidney problems, and any plans for procedures in the near future. UFH is more recommended by the ACF for patients with renal impairment with creatinine clearance <15-30 mL/min. The American Society of Hematology (ASH) states that LMWH or UFH is the

Table 2. Evaluation of inflammatory marker and disease progression (SD: standard deviation).

First to second evaluation		Moderate		Severe		Critically ill with ventilator		P Value
		n=110	P value	n=140	P value	n=81	P Value	
Inflammatory status	1st	40	36.36	73	0.003*	41	50.62	0.32
	2nd	21	19.09	61		48	59.26	
Laboratory evaluation								
Decreasing leucocyte ($10^3/\mu\text{L}$; median, SD) (n, %)	7.3	5.74	51	46.364	0.71	8.46	5.7	0.03*
Decreasing neutrophil (%; median, SD) (n, %)	67.85	26.25	69	62.727	0.004*	77.25	28.22	0.486
Increasing lymphocyte (%; median, SD) (n, %)	16.85	12.05	55	50	0.866	9.65	13.35	0.07
Increasing lymphocyte absolute ($10^3/\mu\text{L}$; median, SD) (n, %)	1.2	0.83	64	58.182	0.16	0.925	1.11	0.544
Decreasing neutrophil-lymphocyte ratio (NLR) (median, SD) (n, %)	3.12	7.98	68	61.818	0.028*	5.67	9.43	0.74
Decreasing C-reactive protein (mg/L; median, SD) (n, %)	17.26	31.429	63	57.273	<0.05*	23.64	70.22	<0.05
Decreasing D-dimer (mcg/L; median, SD) (n, %)	0.51	3.19	58	52.727	0.598	1.362	2.32	0.026
Second to third evaluation		Moderate		Severe		Critically ill with ventilator		P Value
		n=110	P value	n=140	P value	n=81	P Value	
Inflammatory status	2nd	21	19.091	61	<0.05*	48	59.26	0.001*
	3rd	4	3.6364	27		28	34.57	
Laboratory evaluation								
Decreasing leucocyte ($10^3/\mu\text{L}$; median, SD) (n, %)	3.73	4.76	67	60.909	<0.05*	5.57	11.9	0.001*
Decreasing neutrophil (%; median, SD) (n, %)	31.1	35.19	73	66.364	<0.05*	49.05	39.51	<0.05*
Increasing lymphocyte (%; median, SD) (n, %)	10	14.58	31	28.182	<0.05*	3.7	10.77	<0.05*
Increasing lymphocyte absolute ($10^3/\mu\text{L}$; median, SD) (n, %)	0.757	1.067	34	30.909	<0.05*	0.564	2.14	<0.05*
Decreasing neutrophil-lymphocyte ratio (NLR) (median, SD) (n, %)	2.23	4.37	77	70	<0.05*	5.02	7.93	<0.05*
Decreasing C-reactive protein (mg/L; median, SD) (n, %)	2.71	7.83	64	58.182	<0.05*	6.37	15.9	<0.05*
Decreasing D-dimer (mcg/L; median, SD) (n, %)	0.39	0.822	62	56.364	<0.05*	1.078	3.68	<0.05*
First to third evaluation		Moderate		Severe		Critically ill with ventilator		P Value
		P Value	P Value	P Value	P Value	P Value	P Value	
Decreasing leucocyte (%)	69.09	<0.05*	57.86	0.01*	51.85	51.85	51.85	0.60
Decreasing neutrophil (%)	77.27	<0.05*	70.71	<0.05*	65.43	65.43	65.43	<0.05*
Increasing lymphocyte (%)	25.45	<0.05*	30.71	<0.05*	19.75	19.75	19.75	<0.05*
Increasing lymphocyte absolute (%)	34.55	<0.05*	39.29	0.00*	35.80	35.80	35.80	0.013
Decreasing neutrophil-lymphocyte ratio (%)	78.18	<0.05*	70.71	<0.05*	62.96	62.96	62.96	0.135
Decreasing C-Reactive protein (%)	69.09	<0.05*	73.57	<0.05*	72.84	72.84	72.84	<0.05
Decreasing D-dimer (%)	73.64	<0.05*	71.43	<0.05*	76.54	76.54	76.54	<0.05*
Decreasing inflammation status (%)	32.73		32.86		16.05	16.05	16.05	
Days of death (mean)	30.50		11.70		12.95	12.95	12.95	<0.05*
Correlation of inflammation status with mortality (r)	0.337	<0.05**	0.136		0.25	0.25	0.25	0.05**

*p Value from Wilcoxon test.

**p Value from Spearman correlation.

Figure 2. Differentiation of inflammatory parameters following anticoagulants in COVID-19.

Table 3. Odds for inflammation profile on day 1, day 6, and day 13 of anti-coagulant treatment (OR: odds ratio)

Status inflammation	Outcome death					
	Unadjusted OR	95%CI	P Value	Adjusted OR	95%CI	P value
Inflammation 1 st day	1.23	0.98-1.55	0.091	1.29	0.76-2.18	0.34
Inflammation 6 th day	1.59	1.24-2.06	0.001*	2.36	1.46-3.83	<0.05**
Inflammation 13 th day	1.67	1.48-1.89	<0.05*	4.15	2.33-7.42	<0.05**

*p<0.05 from univariate analysis.

**p<0.05 from multivariate regression logistic analysis with adjusted from potential confounder variable.

therapy of choice over oral anticoagulants due to the potential for drug interactions and short half-lives.^{23,24} In our study, the population involved was a group of patients requiring hospitalization due to SARS-CoV2 infection ranging from moderate to critically ill. At all three severity groups, patients receiving anticoagulant therapy had increased D-dimer values. This increase in D-dimers expresses clusters of fibrin lysis and thrombus in the pulmonary vessels. Previously, Guan *et al.* (2020) found that 46% of 1099 COVID-19 patients had an increase in D-dimer and only 5% experienced a decrease in platelet count.^{19,25}

The relationship between coagulation function and indicators of inflammation and infection has been previously analyzed by Long *et al.* (2020). D-dimers were positively correlated with CRP ($r=0.36$, $p=0.0007$) and procalcitonine ($r=0.45$, $p<0.001$).²⁶ Increased CRP was also found in patients with coagulation disorders by Friedrich *et al.* (2020) to a mean level of 131 ± 106 mg/l.²⁷ In our study, there was an increase in CRP by a mean of 40.1 ± 42.43 l in the severe group population receiving anticoagulants that met the criteria for anticoagulant according to The International Society of Thrombosis Haemostasis (ISTH) or due to an increase in D-dimer. A meta-analysis regarding dosing of anticoagulant therapy on the COVID-19 mortality rate found that there was a slight decrease in the mortality rate in COVID-19 patients in need of a ventilator.²⁸ Several studies have shown different results on the mortality outcome of COVID-19 patients receiving anticoagulant therapy. In theory, the coagulation cascade is active when inflammation is present due to SARS-CoV2 infection. Therefore, it may be possible to obtain beneficial effects from the use of anticoagulants as anti-inflammatory agents.

According to the theory of hypercoagulation disorders, there is a two-way relationship between the immune system and thrombin formation, where inhibition of thrombin formation may reduce the inflammatory response.²⁹ The positive effects of using anticoagulants on mortality were reported by Nadkarni *et al.*; compared to non-users, recipients of anticoagulants for both therapy and prophylaxis had reduced mortality (adjusted hazard ratio [aHR] = 0.53; 95% CI: 0.45-

0.62, and aHR = 0.50; 95% CI: 0.45-0.57, respectively), and intubation rates (aHR 0.69; 95% CI: 0.51-0.94, and aHR 0.72; 95% CI: 0.58-0.89, respectively).³⁰ Our study revealed that no reduction in inflammatory markers was significantly correlated with the mortality.

Several other studies have classified the use of anticoagulants according to their intended use for therapeutical and prophylaxis purpose. Klok *et al.* (2020) found that 15% of the population needed ICU while Helms *et al.* (2020) revealed 25 of their patients had pulmonary embolism despite receiving anticoagulant therapy.²² Therefore, anticoagulant prophylaxis is rational. In addition, the prophylactic use of apixaban (odds ratio [OR] 0.46, p=0.001) and enoxaparin (OR=0.49, p=0.001) exhibited a significant decrease in mortality. There was also an association between therapeutic apixaban and decreased mortality rates (OR 0.57, p=0.006). Pawlowski *et al.* (2020) compared the effects of enoxaparin and heparin, showing that patients receiving heparin had a higher risk of death and higher ICU admission than ones in enoxaparin group (risk ratio: 6.76; 95% C.I: [3.39, 12.7]; adjusted p-value <0.0001); (risk ratio of ICU admission: 1.51; 95% C.I: [1.12, 2.03]; adjusted p-value 0.01). Additionally, ICU and hospital length of stay were shorter in the enoxaparin population (mean ICU duration: 0.9 days [standard deviation: 2.5], mean hospital duration: 5.4 days [standard deviation: 4.3]).³¹

In this study, we found favorable changes in inflammatory markers such as white blood cells, neutrophils, lymphocytes, CRP, and D-dimers after the use of anticoagulants. The analysis of the results of this study has been divided based on the subgroup of the severity of COVID-19 disease. From the clinical aspect, analysis of the use of anticoagulants on day of death found significant differences among groups of severity (p<0.05). There was also a significant correlation between inflammatory status on the thirteenth day and mortality based on patient comorbidities obtained from moderate and critical cases on a ventilator (r=0.337; p< 0.05 and r=0.25; p 0.05). Univariate and multivariate analysis exhibited that no reduction in inflammatory profile on day 6 (adjusted odds ratio [aOR]=2.36; 95% CI: 1.46-3.83, p value < 0.05) and day 13 (adjusted odds ratio [aOR]=4.15; 95% CI: 2.33-7.42, p value < 0.05) were linked to the patients' mortality. This study is able to explain that the values of inflammatory and hypercoagulable markers go hand in hand with the severity of the patient. Although not all inflammatory markers improve after anticoagulant treatment, inflammatory variables mostly manifested good results. This study did not distinguish one regimen of anticoagulant from another; thus, it could be a confounder. Conclusively, it is necessary to carry out further subgroup analysis of the types of anticoagulants and the comparison of therapeutical effects and prophylactic use of anticoagulant towards the mortality outcome and length of stay.

Conclusion

Administration of anticoagulants to COVID-19 patients with moderate to critical presentation promoted significant outcomes of inflammatory markers which ultimately showed a statistical difference in mortality. Most of the inflammatory markers in patients improved after anticoagulant administration. Therefore, our findings confirm that the administration of anticoagulants can be optimized since they are able to work as anti-inflammatories.

Data availability Underlying data

figshare: Data of Inflammatory Parameters after Anti-coagulant among Moderate, Severe, and Critically Ill COVID-19 Patients. <https://doi.org/10.6084/m9.figshare.16910905.v2>

This project contains the following files:

- Data_antikoagulan_all_join_3.xlsx (raw data file)
- Legend (Anti-coagulant_311021).docx (data key)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Authors' contributions

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Reviewer Report 09 March 2022

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This is an interesting clinical question relevant to the risk stratification for COVID-19. However, I can find numerous risk stratification studies by utilizing exactly the lab measurements for the disease and there is lack of novelty in the current study. I also have several detailed comments:

1. I do not think the discussion on anticoagulation strategy is relevant for the current study. The current study is a risk factor analysis and the causality of heparin intervention is not explored.
2. The data analysis results described in the abstract do not support the conclusion that "Anticoagulants in COVID-19 patients lower inflammation markers."; the causal inference has not been implemented formally.
3. Many patients would be discharged/dead before day 13, how did you handle such competing risk? In such a situation, death can be regarded as competing risk if you are focusing on inflammation outcomes.
4. "After all the completed researches, further study on how to use anticoagulants is still being analysed to promote the best prognosis."---this discussion is irrelevant to the current study.
5. I suggest that the authors should extract data on heparin use and then explore the causality between heparin and mortality, while stratified by subgroups of population.
6. "We also determined the relationship between inflammatory state after anti-coagulant with patients' mortality, along with some variables, which were age, gender, disease severity, and comorbidity."---this statement is confusing. You need to specify to use multivariable regression model to adjust for confounding. Furthermore, the model specification described here does allow replication of the study. For example, did you consider interaction or non-linearity for the relationship between covariates and outcome? You assumed linearity but may not hold true in real

world data. You need to discuss the limitation of current study in adjusting for confounding factors. However, another approach is to use ensemble modelling¹, which can address non-linearity automatically without pre-specification. At least you need to mention this in the discussion section.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: emergency and critical care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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