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Implications of COVID-19 on Thrombotic Profile of Severely Affected Patients

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Highlights

1. Blood coagulation profile of COVID-19-infected patients is altered.
2. Significant variation is seen in the patients under severe, moderate, and mild categories within each thrombotic profile: platelet, fibrinogen, D-dimer, prothrombin time (PT), and activated partial thromboplastin time.
3. Fibrinogen, D-dimer levels, and PT were significantly elevated, especially in severe and moderately ill COVID-19 patients.
4. Nonsurvivors group had highest mean fibrinogen and D-dimer levels.
5. All measured thrombotic parameters were significantly higher in nonsurvivors than in survivors group of COVID-19 patients.

Keywords
Coronavirus disease 2019 · D-dimer · Fibrinogen · Indian population · Severe acute respiratory syndrome coronavirus 2 · Thrombosis

Abstract

Introduction: Coronavirus disease 2019 (COVID-19) is a novel viral disease that spread as a global pandemic in 2020 by infecting millions of people across the world. Its clinical prognosis is dependent on various coagulatory parameters since thrombotic events are frequently associated with infection severity. ***Methods:*** A total of 383 COVID-19 patients enrolled in Rajiv Gandhi Super Specialty Hospital, Delhi, India, were included in the present retrospective study. Patients were divided into three categories, severe (n = 141), moderate (n = 138), and mild (n = 104) based on infection severity. Various thrombotic parameters and anticoagulant levels were measured in 70 patients and further analyzed. ***Results:*** Coagulopathy is seen in COVID-19 patients (n = 70) with a significant increase in fibrinogen, D-dimer levels, and prothrombin time in patients with severe and moderate disease compared to patients with a mild infection. Approximately, 70% of patients with severe and moderate disease demonstrated fibrinogen levels higher than the standard reference range. 60.41% of patients with severe disease showed significantly higher D-dimer levels. Thrombotic parameters were notably elevated in the nonsurvivors group

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compared to COVID-19 survivors. Nearly, 91% of patients with severe infection had anticoagulant protein S levels below the reference range. ***Conclusion:*** COVID-19 infection severely impacts the blood coagulation cascade, which might lead to the manifestation of severe symptoms and increased mortality in patients.

Introduction

The year 2020 marked the rise and spread of a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) across the world. The infection by SARS-CoV-2 originated in the city of Wuhan, China in late 2019 and was later named coronavirus disease 2019 (COVID-19) by World Health Organization [1, 2]. However, even after the launch of various vaccines to combat this virus, much about the pathophysiology of this disease remains unexplored. In terms of disease manifestation following the COVID-19 infection, a broad-brimmed range of the illness was ascertained, such as asymptomatic or mild to moderate and life-threatening severe infection [1].

Several studies establish that COVID-19 is predominantly a respiratory illness that affects the lungs, is highly associated with coagulopathy [3, 4], and may increase the risk of thrombosis [5]. Dysfunction of the coagulation pathway is related to poor prognosis. Moreover, disseminated intravascular coagulation is prevalent across patients who died from novel coronavirus pneumonia [6].

Many studies have associated increased morality in COVID-19 patients with hypercoagulation and thromboembolic) complications [7–9]. One of the consistent findings is increased D-dimer levels of patients, which is an indicator of intravascular thrombosis [10]. Independent research groups have studied thrombotic rates in COVID-19 patients admitted to ICU with serious complications and those not admitted to ICU with moderate to asymptomatic infection. All the studies reported that incidences of venous thromboembolism were consistently and significantly higher in ICU patients than non-ICU patients [11–13].

Major factors that contribute to thrombogenesis during COVID-19 include dysfunctional endothelium, hyperimmune response with activated platelets, and release of procoagulants, such as plasminogen activator inhibitor (PAI-1) due to activation of the renin-angiotensin-aldosterone system [14]. Since angiotensin-converting enzyme 2 receptor for SARS-CoV-2 is present in endothelial cells [15, 16], the endothelial lining is invaded by this virus, resulting in a severe injury and associated disruption of cellular membranes [17]. Subsequently, endothelial disruption might result in the release of von Willebrand factor, especially in critical patients [18, 19] predisposing thrombus formation [20]. Maier et al. [21] conducted a small study of 15 COVID-19 patients and reported that SARS-CoV-2 virus causes abnormal blood flow due to hyperviscosity. Propagation of thrombus formation is further facilitated by inflammation and cytokine storm during the infection [22].

The present study collects clinical profiles of 383 COVID-19 patients from India. Out of these patients, 251 were males and 132 were females [23]. Thrombotic parameters of 70 patients were recorded and correlated with the severity of infection and mortality. The findings of this study may be helpful in the better clinical management of COVID-19.

Methodology

Data Collection

Clinical data of 383 COVID-19-infected patients' samples were taken from Rajiv Gandhi Hospital, Delhi, India for a retrospective study in the year 2020. The diagnosis of COVID-19 infection was confirmed by RT-PCR, and the patients were grouped under severe, moderate, and mild categories based on the severity of infection and symptoms. Blood samples were collected, and various parameters related to blood coagulation and thrombosis were recorded for 70 patients. The medical reports were analyzed at the Defence Institute of Physiology and Allied Sciences, DRDO, Delhi. Patients were categorized into severe, moderate, and mild cases based on infection severity according to the guidelines laid by the Ministry of Health and Family Welfare, Government of India https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adultsdated24052021.pdf. The data were compiled by a team of scientists and trained clinicians. Patient categorization, along with their demographic data, medication details, and comorbidity status, has been elaborated in Table 1.

Study Design

The collection of samples and data was according to the guidelines laid by the Indian Council of Medical Research (ICMR) and was duly approved by the Ethical Committee. Since the identity of the participants was not disclosed and the analysis of data is retrospective in nature, the written and informed consent from the participants has been waived off.

Blood samples were collected in EDTA and sodium citrate tubes. The samples of the COVID-19-infected subjects, divided into severe, moderate, and mild categories, were analyzed for parameters of coagulation, namely: (1) platelet count, (2) D-dimer, (3) fibrinogen, (4) prothrombin time (PT), (5) activated partial thromboplastin time (APTT), (6) international normalized ratio (INR) and anticoagulants, (7) protein C (PROC), (8) protein S

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Table 1. Basic characteristics of COVID-19 patients along with their medication and comorbidity details

Basic details	Basic characteristics of COVID-19 patients (n = 383)				Basic characteristic of COVID-19 patients having coagulation profile (n = 70)				
	categorization of patients				survivors			nonsurvivors	
	severe (n = 141)	moderate (n = 138)	mild (n = 104)		severe (n = 36)	moderate (n = 8)	mild (n = 2)	n = 24	
Age, years	58	52	43		54	53	51	64	
Gender (M/F)	92/49	99/39	62/43		19/17	5/3	1/1	14/7	
Medication details*									
Plasma therapy	32	8	1		9	NIL	NIL	6	
Tocilizumab	10	2	NIL		3	NIL	NIL	5	
Remedisvir	60	14	2		15	NIL	NIL	18	
Comorbidity details									
Basic details	Severe (n = 141)	Moderate (n = 138)	Mild (n = 104)	p value	Severe (n = 36)	Moderate (n = 8)	Mild (n = 2)	Non-survivors	p value
Diabetes, n (%)	31 (21.9)	17 (12.31)	13 (12.5)	**0.0005**	7 (19.44)	0	1 (50)	5 (20.83)	0.40
Hypertension, n (%)	16 (11.34)	19 (13.76)	10 (9.61)	0.07	5 (13.88)	1 (12.5)	NIL	1 (4.16)	0.81
CVD, n (%)	3 (2.12)	NIL	2 (1.92)	0.13	2 (5.55)	NIL	NIL	NIL	0.73
COPD, n (%)	1 (0.70)	3 (2.17)	2 (1.92)	0.75	NIL	NIL	NIL	NIL	–
Diabetes + hypertension, n (%)	28 (19.85)	19 (13.76)	2 (1.92)	**0.02**	9 (25)	3 (37.5)	NIL	5 (20.83)	0.65
Diabetes + CVD, n (%)	1 (0.70)	2 (1.44)	NIL	0.62	NIL	NIL	NIL	NIL	–
Hypertension + CVD, n (%)	1 (0.70)	6 (4.34)	1 (0.96)	0.06	NIL	1 (12.5)	NIL	NIL	0.18
Diabetes + hypertension + CVD, n (%)	11 (7.80)	5 (3.62)	1 (0.96)	0.77	1 (2.77)	NIL	NIL	7 (29.16)	0.85
Other diseases (hypothyroidism, tuberculosis, etc.), n (%)	6 (4.25)	6 (4.34)	3 (2.88)	0.11	NIL	NIL	NIL	1 (4.16)	–
No comorbidity, n (%)	43 (30.49)	61 (44.20)	70 (67.30)	–	12 (33.33)	3 (37.5)	1 (50)	5 (20.83)	–

CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disorder; LMWH, low molecular weight heparin. * All patients who were on oxygen therapy received anticoagulant, i.e., LMWH. Precisely inj clexane 0.6 mL subcutaneous od/inj clexane 0.4 mL subcutaneous bd was given to moderate and severe COVID-19 patients.

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Table 2. Characteristic details of each data set (severe, moderate, and mild) for coagulation parameters

	Platelet count, lac/mm^3*			Fibrinogen, mg/dL			D-dimer, ng/mL			PT, s			APTT, s			INR		
	severe	mod-erate	mild	severe	mod-erate	mild	severe	mod-erate	mild	severe	mod-erate	mild	severe	mod-erate	mild	severe	mod-erate	mild
Sample size	51	10	7	48	13	7	48	13	7	50	12	8	50	12	8	41	16	12
Lowest value	1.06	1.27	1.45	124	273	292	113	115	141	9.9	10.7	11.4	23.8	24.2	26.2	0.7	0.74	0.83
Highest value	7.34	4.76	3.09	900	754	483	5,000	1,709	5,000	19.6	14.9	12.8	96.3	47.5	36.2	1.74	1.54	1.36
Mean	2.75	2.22	2.004	570.33	490	370.4	1,986.06	486.76	1,197	13.84	12.2	12.087	36.71	33.47	31.92	1.25	1.12	1.06
Std. dev	1.45	1.15	0.66	261.16	145	70.75	1,874.44	455.59	1,885.14	2.25	1.12	0.51	11.87	6.22	3.12	0.25	0.21	0.14
Median	2.45	1.66	1.8	608.5	464	355	1,387	349	189	13.35	12.3	12.25	34.85	33.15	32.3	1.24	1.08	1.05
IQR	1.86	1.35	1.34	507	234.5	143	3,514	482	51.5	3	1.2	0.9	8.8	8.4	8.9	0.38	0.29	0.11
Coefficient of skewness	1.32	1.48	1.1	−0.17	0.4703	0.77	0.50	1.94	1.8	0.80	1.13	−0.23	3.33	10.82	−0.59	0.13	0.28	0.52
	Severe vs. mod-erate	Severe vs. mild	Moderate vs. mild	Severe vs. mod-erate	Severe vs. mild	Moderate vs. mild	Severe vs. mod-erate	Severe vs. mild	Moderate vs. mild	Severe vs. mod-erate	Severe vs. mild	Moderate vs. mild	Severe vs. mod-erate	Severe vs. mild	Moderate vs. mild	Severe vs. mod-erate	Severe vs. mild	Moderate vs. mild
p value	0.22	**0.03**	0.63	0.15	**0.0001**	**0.02**	**0.000005**	0.33	0.36	**0.0009**	**0.00001**	0.76	0.20	**0.02**	0.47	0.06	**0.001**	0.37

* For conversion to SI unit 10^9/L: Multiply the numeric part by 100.

Table 3. Percentage of patients (severe, moderate, and mild) above reference range, within reference range and below reference range (compared to standard reference range) for each coagulation parameter and anticoagulant

Thrombotic parameters	Standard ref range	Severe			Moderate			Mild			*p* value		
		above ref range, *n* (%)	within ref range, *n* (%)	below ref range, *n* (%)	above ref range, *n* (%)	within ref range, *n* (%)	below ref range, *n* (%)	above ref range, *n* (%)	within ref range, *n* (%)	below ref range, *n* (%)	severe vs. mild	severe vs. moderate	mild vs. moderate
Platelet count	1.5–4.50 lacs/mm^3*	6 (11.76)	40 (78.43)	5 (9.8)	1 (10)	7 (70)	2 (20)	0	7 (100)	0	0.39	0.65	0.28
Fibrinogen	175–400 mg/dL	33 (68.75)	11 (22.91)	4 (8.33)	9 (69.23)	4 (30.76)	0	2 (28.57)	5 (71.42)	0	0.51	0.51	–
D-dimer	0–500 ng/mL	29 (60.41)	19 (39.58)	0	3 (23.07)	10 (76.92)	0	2 (28.57)	5 (71.42)	0	–	–	–
PT	10.4–16.6 s	5 (10)	44 (88)	1 (2)	0	12 (100)	0	0	8 (100)	0	0.59	0.45	–
APTT	24.9–38.2 s	15 (30)	32 (64)	3 (6)	2 (16.66)	9 (75)	1 (8.3)	0	8 (100)	0	0.12	0.64	0.31
INR	<1.0–2.0	0	41 (100)	0	0	16 (100)	0	0	12 (100)	0	–	–	–
PROC	15–150%	0	11 (100)	0	0	1 (100)	0	0	1 (100)	0	–	–	–
PROS	70–130%	0	1 (9.09)	10 (90.90)	1 (100)	0	0	1 (100)	0	0	**0.002**	**0.002**	–
AT	79–125%	1 (9.09)	8 (72.72)	2 (18.18)	0	1 (100)	0	0	0	1 (100)	0.19	0.83	–

* For conversion to SI unit 10^9/L, multiply the numeric part by 100.

Table 4. Average value of coagulation parameters in survivors and nonsurvivor patients compared to standard reference range

	Std. min range	Average (mean) value in survivors (n = 46)	Average (mean) value in nonsurvivors (n = 24)	Std. max range	p value
Platelet count, lacs/mm^3*	**1.5**	2.51	1.94	**4.5**	**0.024**
Fibrinogen, mg/dL	**175**	461.50	579.42	**400**	**0.0429**
D-dimer, ng/mL	**0**	1,204.43	2,199.62	**500**	**0.0312**
PT test, s	**10.4**	13.11	14.54	**16.6**	**0.0131**
APTT, s	**24.9**	32.63	39.53	**38.2**	**0.0212**
INR	**1**	1.12	1.26	**2**	**0.0240**

* For conversion to SI unit 10^9/L, multiply the numeric part by 100.

(PROS), and (9) antithrombin (AT). Blood samples were drawn at the time of hospital admission. However, for severe patients who were admitted to the hospital for a longer duration, all coagulatory parameters were monitored at regular intervals. In the present study, an average value of parameters (applicable to repeated test values) was utilized for statistical analysis.

Whole blood was collected in EDTA tubes for the measurement of platelet count by an electrical impedance method using a Coulter LH750 Hematology Analyzer (Beckman Coulter, Brea, CA, USA). Platelet count was measured in lacs/cu.mm. (For conversion to SI unit 10^9/L, multiply the numeric part by 100.) To measure D-dimer (agglutination assay), fibrinogen, PT, APTT, PROC, PROS (mechanical clot detection method), and AT (optical cloth method), a medium-throughput analyzer, DESTINY PLUS (Tcoag, Ireland), was used.

Statistical Analysis

The statistical analysis was performed using MEDCALC (MedCalc Software Ltd., Ostend, Belgium) statistical software. The coagulation parameter details (platelet count, fibrinogen, D-dimer, PT, APTT, and INR), such as the number of samples in each category (severe, moderate, and mild) along with the lowest value, highest value, mean, median, standard deviation, and coefficient of skewness, were calculated. Anticoagulants were not detailed in this manner due to the small sample size. The average percentage value of thrombotic parameters and anticoagulants in each category of patients was compared with the standard reference range of the respective parameter. The subjects were divided into three categories, with a parameter above, within, and below the reference range. For estimation of statistically significant difference (p value), the χ^2 test (Tables 1, 2) and Welch's t test (Tables 3, 4) were used.

Results

All COVID-19 patients (n = 383) were categorized into severe (n = 141), moderate (n = 138), and mild (n = 104) forms of infection. The proportion of COVID-19-infected males was relatively higher than those of infected females (Table 1). It was further observed that the number of older patients was higher in the nonsurvivor group (Table 1). Out of 383 COVID-19 patients, we were able to obtain and analyze coagulation parameters of only 70 patients (as elaborated in Table 1), namely the platelet count, fibrinogen, D-dimer, PT, and APTT. We were also able to obtain anticoagulant data comprising PROC, PROS, and AT for a few patients (Fig. 1).

Medication Administered to COVID-19 Patients

Details of treatment provided to COVID-19 patients have been described in Table 1. Besides antiviral therapy, anticoagulants were also given to patients under moderate and severely infected category according to the ICMR guidelines, Government of India. Low molecular weight heparin, inj clexane 0.6 mL once a day, was administered subcutaneously to all moderate and severely infected patients. Among these patients, 8 severe patients and 4 moderate patients were on anticoagulants (Ecosprin 75 mg) prior to their admission to the hospital.

Comorbidity Details of COVID-19 Patients

The comorbidity status of study subjects has been detailed in Table 1. Diabetes mellitus and hypertension were observed to be predominantly associated with COVID-19 infection severity. Moreover, the percentage of patients suffering from greater than one comorbidity was higher in severe and nonsurvivor groups than patients with moderate and mild infections. On the other hand, those without any comorbidity mainly belonged to the moderate and mild categories (Table 1). Patients with diabetes as well as those with diabetes along with hypertension were significantly higher in the severe group than moderate and mild patients (p = 0.005 and p = 0.02, respectively), as illustrated in Table 1.

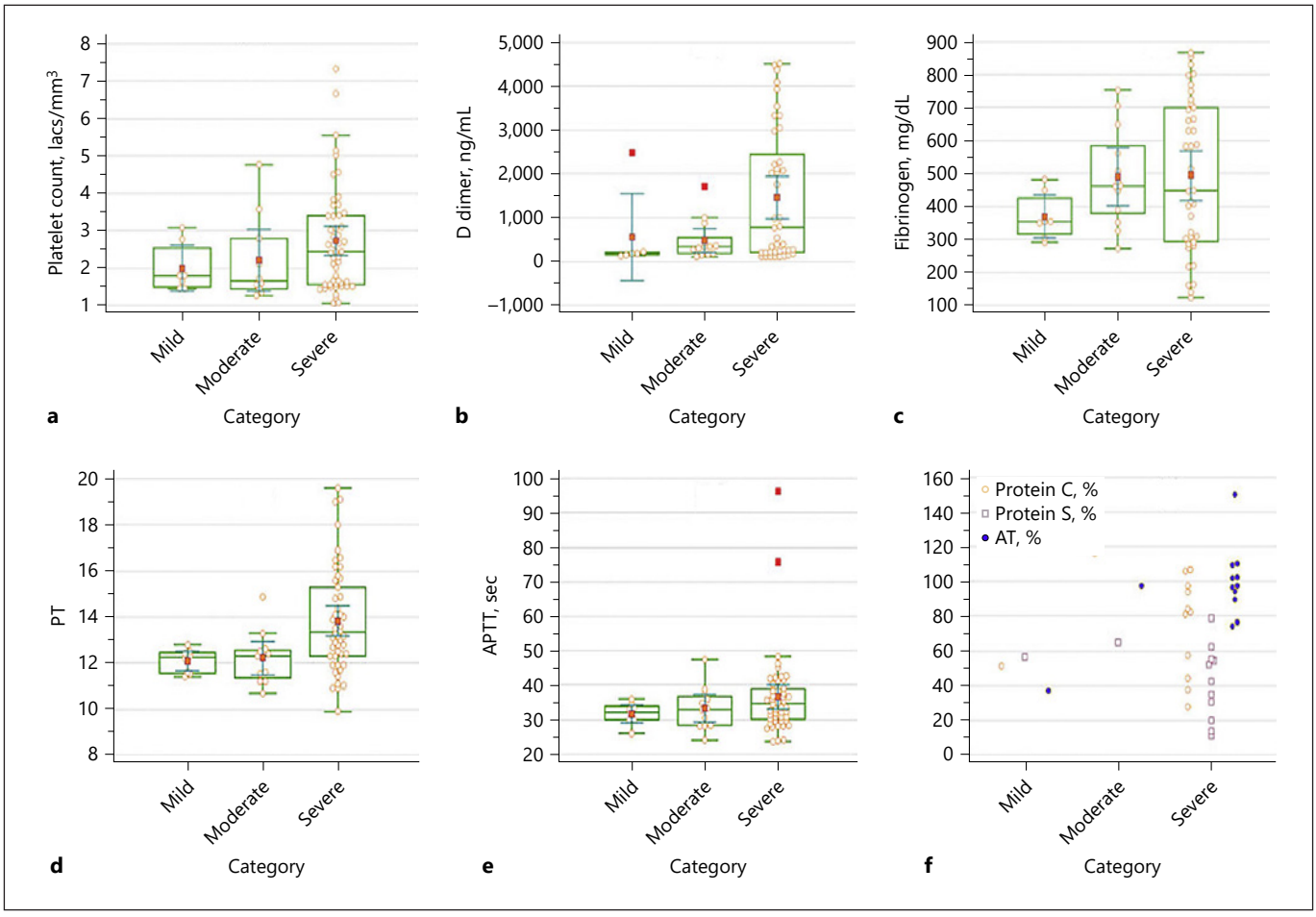


Fig. 1. Box and Whisker plot of COVID-19-infected patients under severe (n = 51), moderate (n = 13), and mild (n = 7) categories for coagulation parameters. Platelet count (**a**), D-dimer (**b**), fibrinogen (**c**), PT (**d**), APTT (**e**), and anticoagulants: PROC, PROS, and AT (**f**).

COVID-19 Infection and Parameters of Coagulation

Six most common blood coagulation (thrombotic) indicators were noted for each patient, viz., platelet count, D-dimer, fibrinogen, PT, APTT, and INR. Platelet count (standard reference range 1.5–4.5 lacs/mm^3) showed a significant difference among the patients with severe and mild disease (p = 0.03). However, no significant change was observed between severe and moderate patients (p = 0.22) as well as between moderate and mild patients (p = 0.63). The mean platelet count was highest in severe patients (2.45 lacs/mm^3) and declined in moderately infected patients (2.22 lacs/mm^3) and patients with mild illness (2.004 mm^3) (Table 3). Interestingly, 78.43% severely infected patients, 70% of moderately infected persons, and 100% of mild patients reported a platelet count within the normal range (Table 2).

Furthermore, the clotting ability indicator, mean fibrinogen content (reference range 175–400 mg/dL), was higher than the standard reference range in severe (570.33 mg/dL) and moderately ill patients (490 mg/dL), although the difference among the two groups was statistically insignificant (p = 0.15) (Table 3). This above-normal trend was seen in 68.75% severe patients and in 69.23% moderate patients (Table 2). Comparing patients with severe and moderate disease to patients with mild disease, the fibrinogen levels were significantly lower in the mild group (p = 0.0001 and p = 0.02, respectively, Table 3). Moreover, a fibrin blood clot degradation product, D-dimer, was elevated in patients with severe disease relative to patients with moderate disease (p = 0.000005, Table 3). D-dimer levels were higher than the normal reference range in 60.41% of severe COVID-19 patients with a mean value of 1,986.06 ng/mL (Table 2).

An abnormal or extended PT, which is an indicator of bleeding disorders, and APTT assay, which is a universally accepted screening procedure used to detect abnormality in the intrinsic coagulation system, were also measured among the three groups of the patient population. PT was significantly higher in patients with severe disease than patients with moderate and mild disease ($p = 0.0009$, $p = 0.00001$, respectively, Table 3). Moreover, APTT showed a significant elevation in severe patients compared to mild patients ($p = 0.02$, Table 3). Approximately, PT of 88% of severe patients and 100% of moderate and mild patients were within the normal range. Similarly, 64% of severe patients, 75% moderate patients, and 100% of mild patients had their APTT within the normal range (Table 2).

INR was statistically significant in severe patients in comparison with mild patients ($p = 0.001$, Table 3). However, all patients showed INR values within the normal reference range (Table 2).

COVID-19 Infection and Anticoagulants

Data available for anticoagulants, PROC, PROS, and AT, was available for relatively fewer numbers of patients. Although PROC levels were within the normal reference range for all severe patients ($n = 11$), an interesting observation demonstrated that PROS levels were lower than the normal reference range in 10 out of 11 severely infected patients (Table 2). AT levels were also within the normal range in 8 out of 11 patients, whereas 2 patients had AT levels below the normal range (Fig. 1).

COVID-19 Infection in Nonsurvivors Compared to Survivor Group

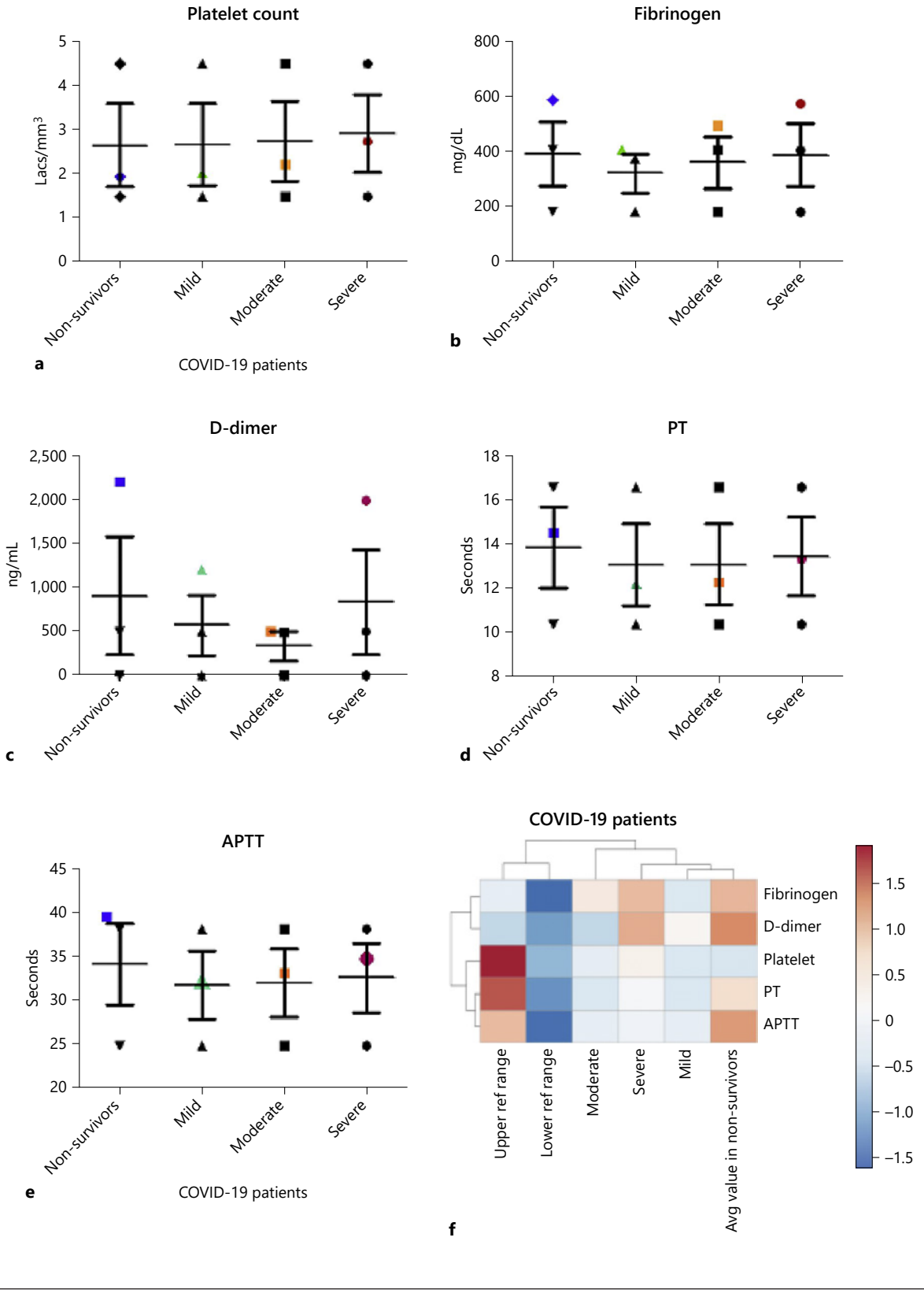
Twenty-four patients out of 383 were deceased (6.26%) [23]. Out of these, coagulation parameters were recorded multiple times in 70 patients. The average fibrinogen levels in the nonsurvivors were 579.42 mg/dL (against the maximum standard upper limit of 400 mg/dL). The average D-dimer level in these patients was as high as 2,199.62 ng/mL (against the standard upper limit of 500 ng/mL). For the other forty-six survivors, for which thrombotic profile was available, the average fibrinogen levels were 461.50 mg/dL, while the average D-dimer was 1,204.43 ng/mL. Other parameters were within the standard reference range (Fig. 2a–e; Table 4). Notably, all six thrombotic parameters measured in the present study showed statistically significant differences among the survivors and nonsurvivors (Table 4). Figure 2f depicts the heat map of coagulation parameters in all study groups, depicting a relationship between thrombotic factors (fibrinogen, D-dimer, platelet, APTT, PT) and COVID-19 patients (mild, moderate, severe).

Discussion

The activated endothelial response during COVID-19 infection is considered to be the major driver for microvascular dysfunction and thrombosis. Platelet recruitment and activation occur as a result of disruption of the endothelial lining, which exposes the collagen-containing subendothelial matrix [24]. Activated platelets undergo degranulation and aggregation to form platelet plugs, where the adhesion of coagulation factors occurs [24]. Although a steady increase in the mean platelet value from mild, moderate to severe patients was seen in our study, the values remained well within the standard reference range (Table 3). Platelet levels were significantly higher in severe patients than in mild COVID-19 patients. The present findings are supported by similar studies of platelet aggregation during COVID-19 infection [25, 26]. In addition, Zhang et al. [27] reported COVID-19 infection-induced platelet activation via binding of angiotensin-converting enzyme 2 spike, which may be further involved in thrombus formation. However, several contradictory reports show thrombocytopenia (low platelet count) during COVID-19 infection [28]. The data available also indicate an association of thrombocytopenia with abnormal coagulation function and disseminated intravascular coagulation, which leads to COVID-19 severity [29, 30].

Dysregulated immune system, decreased fibrinolysis, and increased coagulation create a prothrombotic milieu during COVID-19 infection. Activated coagulation cascade converts fibrinogen to fibrin, which forms blood clots (thrombi) as well as platelet aggregates [31]. The presence of fibrinogen is, therefore, an important determinant of blood viscosity, and thus, its elevated levels are recorded in COVID-19 patients [32, 33]. We found a significant increase in fibrinogen levels in severe and moderate patients in our data.

Progression of COVID-19 infection marks a reduction in fibrinolysis and abnormalities in clot dissolution [33]. A fibrinolytic pathway inhibitor, PAI-1, increases during COVID-19, SARS-CoV-1 infection, and acute respiratory distress syndrome, which results in the hypofibrinolysis and fibrin deposition [34]. Inflammation during the infection further enhances PAI-1 release from endothelial cells, which suppresses two plasminogen activators,



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urokinase plasminogen activator, and tissue plasminogen activator, thus blocking the conversion of plasminogen to plasmin, thereby diminishing fibrin degradation [35]. Incomplete breakdown of intravascular thrombus in venous thrombosis results in increased intermediate degradation product like D-dimer levels in the blood. Furthermore, a case study reports that the intra-alveolar fibrin breakdown also increases D-dimer levels, resulting in acute lung injury in COVID-19-infected individuals [18].

Under present study, for statistical analysis, an average value of all parameters (applicable to repeated test values) was considered. Clinical data showed a marked elevation in D-dimer levels in severe COVID-19 patients (Table 3). This sharp increase in D-dimer values has been associated with increased mortality [36, 37]. In line with the previous reports, our study also demonstrates the highest D-dimer levels in deceased patients. The higher levels of mean D-dimer seen in the mild patients are due to a few outlier samples.

Another coagulopathy indicator, PT, was significantly higher in patients with severe and moderate infection than in patients with mild infection. This observation is in concurrence with the recent finding of Long and coworkers, who stated that both D-dimer and PT levels may be used as a potential indicator for predicting mortality due to COVID-19 infections [38]. We also observed a significant increase in APTT and INR levels while comparing severely infected patients to mild COVID-19 patients. However, all patient groups fell within the normal reference ranges for PT, APTT, and INR.

A vascular endothelial injury might also reduce the level of anticoagulants in the blood. Massberg et al. [39] reported that action of tissue factor is enhanced upon degradation of endogenous coagulants, thereby promoting clot formation. In the present study, data for anticoagulants were available for very limited samples; however, an interesting observation showed a reduced PROS in severely ill patients. These observations are in accordance with other studies, which stated that deficiency of PROS in COVID-19 patients is associated with disease severity and could lead to thrombotic manifestations of the disease [40–42].

Fig. 2. Groupwise distribution of coagulation parameters in nonsurvivors (24), mild (7), moderate (13), and severe (51) COVID-19 patients. Platelet count (**a**), fibrinogen (**b**), D-dimer (**c**), PT (**d**), and APTT (**e**). Black dots correspond to the upper and lower reference range, whereas colored dots correspond to the average value of the respective parameter. **f** Heat map of average coagulation parameters in different patients' groups.

Although the association of COVID-19 and thrombosis is being actively investigated, the phenomenon of venous thrombosis and its devastating consequences requires a more comprehensive understanding to develop better treatment strategies and mitigate the effect of COVID-19 infection. Data presented in this study depict that clinicians should be aware that if thrombotic parameters, such as fibrinogen and D-dimer, are increased beyond reference range in COVID-19 patients, there is a need to reevaluate their thromboembolic risk as well as to reconsider the dosage of anticoagulation as per ASH guidelines [43].

Conclusion

COVID-19 is associated with frequent thrombotic events, which increase disease severity and result in poor prognosis. Levels of thrombotic markers, such as fibrinogen and D-dimer, must be constantly monitored in COVID-19 patients by clinicians, in order to recommend appropriate anticoagulation treatment along with antiviral medication.

Statement of Ethics

The collection of samples and data was according to the guidelines laid by ICMR and was duly approved by Institutional Ethics Committee, Rajiv Gandhi Super Specialty Hospital (approval number RGSSH/01/2020/03). Since the identity of the participants has not been disclosed and the analysis of data is retrospective in nature, the written and informed consent from the participants has been waived off.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Iti Garg: conceptualization, writing—original draft preparation, reviewing and editing, and formal analysis. Swati Srivastava: conceptualization, writing—original draft preparation, reviewing and editing, and formal analysis. Vikas Dogra: method-

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Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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