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Prothrombotic changes in patients with COVID-19 are associated with disease severity and mortality

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Abstract

Background and Aims: Patients with severe coronavirus disease 2019 (COVID-19) are at significant risk of thrombotic complications. However, their prothrombotic state is incompletely understood. Therefore, we measured in vivo activation markers of hemostasis, plasma levels of hemostatic proteins, and functional assays of coagulation and fibrinolysis in plasma from patients with COVID-19 and determined their association with disease severity and 30-day mortality.

Methods: We included 102 patients with COVID-19 receiving various levels of respiratory support admitted to general wards, intermediate units, or intensive care units and collected plasma samples shortly after hospital admission.

Results: Patients with COVID-19 with higher respiratory support had increased in vivo activation of coagulation and fibrinolysis, as reflected by higher plasma levels of D-dimer, thrombin-antithrombin, and plasmin-antiplasmin complexes as compared to patients with no to minimal respiratory support and healthy controls. Moreover, the patients with COVID-19 with higher respiratory support exhibited substantial ex vivo thrombin generation and lower ex vivo fibrinolytic capacity, despite higher doses of anticoagulant therapy compared to less severely ill patients. Fibrinogen, factor VIII, and von Willebrand factor levels increased, and ADAMTS13 levels decreased with increasing respiratory support in patients with COVID-19. Low platelet count; low levels of prothrombin, antithrombin, and ADAMTS13; and high levels of von Willebrand factor were associated with short-term mortality.

Conclusions: Severe COVID-19 is associated with prothrombotic changes with increased in vivo activation of coagulation and fibrinolysis, despite anticoagulant therapy.

KEYWORDS

coagulation, COVID-19, fibrinolysis, hemostasis, thrombosis

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Essentials

- Thrombotic complications are common in patients with coronavirus disease 2019 (COVID-19).
- We assessed hemostatic parameters in relation to outcome in 102 patients with COVID-19.
- High respiratory support and mortality were associated with prothrombotic changes.
- Hyperactivation of coagulation might play a role in progression of lung injury in COVID-19.

1 | INTRODUCTION

Patients with coronavirus disease 2019 (COVID-19) are at increased risk of venous thromboembolism (VTE).[1] In particular, patients with COVID-19 admitted to intensive care units (ICUs) exhibit high incidences of VTE (approximately 10%-30%)[2-4] compared to patients with COVID-19 in general wards (VTE incidences of approximately 6%).[5-7] Notably, most of these patients were receiving standard- or higher-dose thromboprophylactic therapy at the time of VTE diagnosis. The most commonly reported thrombotic complication is pulmonary embolism (PE), with a 5- to 6-fold higher incidence in patients with COVID-19 compared to the general ICU population on thromboprophylaxis.[8] A recent cohort study reported an absolute incidence of 20% of PE in 107 consecutive patients with COVID-19 admitted to the ICU compared to 6.1% and 7.5% in a matched general ICU patient cohort and influenza-positive ICU cohort, respectively, admitted to the same ICU a year earlier.[9] Importantly, postmortem studies have demonstrated abundant microthrombi in pulmonary and extrapulmonary vascular beds[10-12] that contain fibrin, platelets, neutrophils, and neutrophil extracellular traps (NETs).[13] These thrombi have been proposed to contribute to lung injury, progression of disease, and multiple organ failure. Given that symptomatic deep vein thrombosis (DVT) is much less commonly observed than PE in patients with COVID-19,[2] it has been proposed that the thrombotic events are in fact not emboli but rather primary pulmonary thromboses.[14]

The pathophysiological mechanisms related to the increased risk of thrombotic complications in patients with COVID-19 are incompletely understood. It has been postulated that the massive activation of the immune system in response to COVID-19 and the associated cytokine storm results in endothelial damage, formation of NETs, platelet activation, and hyperactivation of coagulation.[13,15] Specifically, activation of endothelial cells by the cytokine storm and possibly by direct infection with severe acute respiratory syndrome coronavirus 2 might play an important role in dysregulation of hemostasis by increasing vascular permeability and promoting recruitment, binding, and activation of immune cells.[16,17] The hemostatic status of patients with COVID-19 has not been studied extensively, but alterations in hemostatic proteins seem more profound with increasing severity of disease.[18,19]

Clinical studies have reported abnormalities in conventional coagulation tests, such as a prolonged prothrombin time (PT) and thrombocytopenia in a proportion of patients, that were associated with increased disease severity.[15] In addition, extremely high levels of D-dimer and other fibrin degradation products have been found in patients with COVID-19 and have been associated with death.[20,21] Several studies demonstrated a hypercoagulable profile by whole blood thromboelastography with conflicting results on the presence of characteristics of disseminated intravascular coagulation.[22,23] Moreover, recent reports demonstrated significant ex vivo thrombin generation in the majority of patients with COVID-19 despite anticoagulant therapy, suggesting that before anticoagulation, patients are profoundly hypercoagulable.[18,19,24] This hypercoagulable state is also detected in rotational thromboelastography studies using the EXTEM, which contains a heparin-neutralizing agent.[25] In addition, a hypofibrinolytic state has been demonstrated using whole blood thromboelastography, tissue plasminogen activator–modified thromboelastography, and a plasma-based clot lysis test.[18,19,26]

Here, we assessed the hemostatic status of 102 patients with COVID-19 on admission in relation to disease severity based on level of respiratory support and level of care and 30-day mortality. We assessed the hemostatic status of these patients by measuring in vivo activation markers of hemostasis, plasma levels of hemostatic proteins, and functional assays that determine ex vivo thrombin generation and fibrinolytic capacity.

2 | MATERIALS AND METHODS

2.1 | Study population

We prospectively included 102 patients with COVID-19 admitted to Danderyd Hospital, Stockholm, Sweden, between April 9 and June 8, 2020. All patients were diagnosed with COVID-19 based on reverse-transcriptase polymerase chain reaction (RT-PCR) viral RNA detection of nasopharyngeal or oropharyngeal swabs or clinical presentation. Inclusion was conducted consecutively provided that research personnel were available. Exclusion criteria were age < 18 years, and four patients were diagnosed with VTE (three PEs, one DVT) before blood sampling and received full-dose anticoagulant treatment, and were therefore excluded from this study. Demographic data, comorbidities, medications, and clinical variables including respiratory support and 30-day mortality were obtained from medical records. Patients were divided into groups based on respiratory support at the time of blood sampling (no respiratory support, ≤5 L of oxygen on nasal cannula or mask, and higher respiratory support that comprised > 5 L of oxygen on nasal cannula or mask, noninvasive respiratory support, and intubation). Level of respiratory support and oxygen concentration were set at the discretion of the treating physician. Thirty-day mortality was

defined as mortality within 30 days from admission to the hospital. Notably, 16 patients (18%) admitted to the general ward did not receive anticoagulants at the time of blood sampling, since these patients were included early in the COVID-19 pandemic, and clear hospital guidelines on enhanced anticoagulant treatment in this patient population had not yet been established. As shown in Table 1, the majority of patients (62%) admitted to the general ward received standard prophylactic low-molecular-weight heparin (LMWH)

TABLE 1 Demographic, clinical and routine laboratory data of study participants

Variable	General ward (n = 90)	High care (n = 12)[a]	*P*-value	No respiratory support (n = 38)	Nasal cannula/ mask ≤ 5L O_2 (n = 46)	Higher respiratory support[b] (n = 18)	*P*-value
Age	60 (50-70)	57 (51-66)	.533	56 (39-68)	60 (51-69)	62 (53-69)	.3
Female	33 (36.7)	4 (33.3)	.822	16 (42.1)	16 (34.8)	5 (27.8)	.558
BMI, kg/m^2	27.8 (24.6-31.6)	27.7 (24.2-31.5)	.932	26.3 (24.0-29.7)	28.4 (25.1-32.4)	28.4 (25.9-31.0)	.238
Oxygen requirement, L/min	1.0 (0.0-3.0)	8.0 (3.0-11.0)	.001	0.0 (0.0-0.0)	2.0 (1.0-3.8)	8.0 (6.0-10.0)	<.001
Comorbidity on admission							
Cardiovascular	14 (15.6)	4 (33.3)	.129	4 (10.5)	8 (17.4)	6 (33.3)	.112
Diabetes	24 (26.7)[c]	2 (16.7)	.455	8 (21.1)	16 (34.8)	2 (11.1)	.108
Renal dysfunction	10 (11.1)	0 (0.0)	.224	2 (5.3)	8 (17.4)	0 (0.0)	.054
Symptom duration, days	10 (6-14)	14 (9-16)	.146	9 (6-14)	11 (7-14)	13 (9-15)	.144
Days between admission and blood sampling	2 (2-3)	2 (2-6)	.238	2 (2-3)	2 (2-3)	3 (2-5)	.021
Routine laboratory values							
Creatinine, mg/dL	73 (58-91)	73 (48-86)	.365	78 (64-92)	72 (57-90)	73 (54-90)	.892
CRP, mg/dL	93 (58-163)	204 (107-279)	.008	75 (24-121)	115 (81-170)	197 (92-289)	<.001
Lactate, mg/dL	1.3 (1.1-1.8)	1.5 (1.1-2.2)	.525	1.3 (1.1-2.1)	1.3 (1.1-1.8)	1.4 (1.0-1.7)	.948
WBC count, $\times 10^9/L$	5.8 (4.4-7.8)	10.0 (6.8-11.1)	.003	5.7 (4.2-7.9)	5.8 (4.5-7.7)	8.8 (6.4-11.2)	.005
Anticoagulation at time of blood sampling			<.001				<.001
No	16 (17.8)	0 (0.0)		10 (26.3)	6 (13.0)	0 (0.0)	
Standard prophylactic LMWH (4500 IU once daily)	56 (62.2)	2 (16.7)		21 (55.3)	32 (69.6)	5 (27.8)	
Intermediate prophylactic LMWH (4500 IU twice daily)	14 (15.6)	10 (83.3)		6 (15.8)	6 (13.0)	12 (66.7)	
OAC	4 (4.4)	0 (0.0)	.456	1 (2.6)	2 (4.3)	1 (5.6)	.853
Anti-Xa, U/mL	0.04 (0.01 -0.11)	0.10 (0.04-0.26)	.046	0.03 (0.00-0.09)	0.04 (0.01-0.10)	0.12 (0.05-0.26)	.003
Outcome							
Deceased[d]	6 (6.7)	4 (33.3)	.004	1 (2.6)	4 (8.7)	5 (27.8)	.012
Discharged alive	84 (93.3)	8 (66.7)	.002	38 (100)	42 (91.3)	12 (66.7)	.006
Still in hospital	1 (1.1)	0 (0.0)		0 (0.0)	0 (0.0)	1 (5.6)	
Duration of hospital stay, days	5 (3-10)	11 (8-17)	.001	3 (2-6)	6 (4-11)	12 (7-17)	<.001

Note: The results are presented as median (interquartile range) for continuous variables and number (percentage) for categorical variables. Comparisons between the two groups are made using the Mann-Whitney U test or Fisher's exact test, as appropriate.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; LMWH, low-molecular-weight heparin; OAC, oral anticoagulant; WBC, white blood cell.

[a]Three patients were admitted to the intensive care unit, and nine patients were admitted to the intermediate care unit.

[b]Respiratory support in this group comprised > 5 L O_2 by nasal cannula/mask (n = 14), noninvasive ventilation (n = 2), and intubation (n = 2).

[c]One patient had type 1 diabetes.

[d]All deaths were due to complications of COVID-19.

(4500 IU once daily), 14 patients (16%) received double standard prophylactic doses of LMWH (4500 IU twice daily), and four patients (4%) received oral anticoagulants. All patients admitted to higher-level care units received anticoagulation (2 received 4500 IU LMWH once daily, and 10 received 4500 IU LMWH twice daily).

Plasma samples from 29 healthy individuals were obtained to establish reference values for the various assays performed. The healthy control group, of whom 35% were female, had a median age of 60 (28-70) years and had a median body mass index of 22.5 (24.8-26.6) kg/m^2. The plasma samples of the healthy controls were obtained from a previous study (dnr 2015/1533-31/1) for which healthy individuals were recruited through local advertisements and/or word of mouth. Exclusion criteria were (chronic) disease and medication use, with the exception of hypertension and blood pressure medication. However, samples in both studies were collected by the same research nurse using the same protocol for sample processing. The study complied with the Declaration of Helsinki, and informed consent was obtained from all healthy individuals and patients, or in the case of incapacity, their next of kin. The protocol was approved by the Stockholm Ethical Review Board (COMMUNITY study, dnr 2020-01 653).

2.2 | Study procedures

Blood samples were collected up to 7 days (median, 2 [2-3] days) after hospital admission. Peripheral blood was drawn either via venipuncture or from preexistent arterial lines into 3.2% sodium citrate (9:1 vol/vol) vacuum tubes. Platelet-poor plasma was prepared from citrated whole blood following centrifugation within 2 hours from sampling for 20 minutes at 2000 *g* at room temperature and stored at − 80˚C until further analyses. Plasma preparation from patients and healthy individuals was performed by the same research nurse following the same protocol. Notably, the time between blood sampling and last dose of anticoagulation was not standardized.

2.3 | Assays

Platelet and white blood cell (WBC) counts, C-reactive protein (CRP), and creatinine and lactate assays were performed by the Laboratory of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden, as part of routine clinical care. All remaining analyses were performed at the University Medical Center Groningen, the Netherlands. Routine hemostatic tests, including PT, fibrinogen, prothrombin, antithrombin, factor VIII, and D-dimer were performed on an automated coagulation analyzer (STACompact 3, Stago, Breda, the Netherlands) with the use of reagents and protocols from the manufacturer. Plasma levels of von Willebrand factor (VWF) were determined using an in-house ELISA with commercially available polyclonal antibodies against VWF (Dako, Glostrup, Denmark). ADAMTS13 activity in plasma was measured using the fluorescence resonance energy transfer (FRETS)-VWF73 assay (Peptanova GmbH, Sandhausen, Germany). Plasminogen activator inhibitor type 1 (PAI-1) levels were quantified using a commercially available ELISA from R&D Systems (Minneapolis, MN, USA). Thrombin-antithrombin (TAT) complex levels were measured using a commercially available ELISA (Siemens, Berlin, Germany). Quantification of plasmin-antiplasmin (PAP) complex levels was performed with the use of a commercially available ELISA (Technozyme; Technoclone, Vienna, Austria).

Thrombomodulin-modified thrombin generation assay was performed with the fluorimetric method calibrated automated thrombinography, as described previously by Hemker et al.[27] In short, coagulation was activated using commercially available reagents (Thrombinoscope, Maastricht, the Netherlands) containing recombinant tissue factor (final concentration, 5 pM), phospholipids (final concentration, 4 μM), and soluble thrombomodulin (final concentration not disclosed by manufacturer). Assays were performed following the manufacturer's instructions, with the exception of a centrifugation at 10 000 *g* for 10 minutes of plasma after it was thawed as described previously.[28] We analyzed the following parameters: endogenous thrombin potential (ETP), representing the total enzymatic work performed by thrombin during the time that it was active; peak (thrombin); lag time, defined as the time to reach one-sixth of the peak thrombin concentration; and velocity index (slope between end of lag time and peak thrombin).

Anti-Xa levels were determined on an automated coagulation analyzer (STACompact 3, Stago) using Heparin LRT (HYPHEN BioMed, Nodia, Amsterdam, the Netherlands).

Fibrinolytic capacity in plasma was assessed by monitoring changes in turbidity during clot formation and lysis of a tissue factor–induced clot by exogenous tissue plasminogen activator, as described previously.[29] Clot lysis time was defined as the time from the midpoint of the clear to maximum turbid transition, representing clot formation, to the midpoint of the maximum turbid to the clear transition, representing clot lysis.

2.4 | Statistical analyses

Data are presented as median (interquartile range) for continuous variables and number (percentage) for categorical variables. Comparisons of hemostatic tests between patients with COVID-19 and healthy controls or COVID-19 patient subdivisions into two groups were made using the Mann-Whitney *U* test or Student *t* test, as appropriate. Comparisons between multiple groups were done with the use of the Kruskal-Wallis test with Dunn's post hoc test. No corrections were made for multiple hypothesis testing. Statistical analyses were performed using SPSS Statistics, version 26 (IBM Inc., Armonk, NY, USA) and Prism (GraphPad Software Inc., La Jolla, CA, USA). A *P*-value of $< .05$ was considered statistically significant.

TABLE 2 Hemostasis tests in patients with COVID-19 and healthy controls with additional subdivision in patients with COVID-19 that were admitted to a ward or to an intermediate-intensive care unit (high care) at time of blood sample

	Healthy controls (n = 29)	Patients with COVID-19 (n = 102)	Patients with COVID-19 General ward (n = 90)	Patients with COVID-19 High care (n = 12)	*P*-value Patients vs Controls	*P*-value Ward vs High care
Standard hemostasis tests						
PT, s	13.9 (13.5-14.6)	15.3 (14.2-16.4)	15.3 (14.1-16.3)	15.8 (14.9-16.8)	<.0001	.309
Platelet count, $\times 10^9$/L		231 (166-328)	229 (171-324)	262 (166-404)		.398
Additional hemostasis tests						
Factor VIII, %	136 (114-157)	219 (159-272)	218 (159-271)	231 (165-298)	<.0001	.533
VWF, %	108 (83-128)	356 (248-442)	348 (245-437)	425 (321-465)	<.0001	.143
ADAMTS13, %	85 (72-96)	68 (50-82)	70 (52-82)	57 (42-62)	.002	.046
Prothrombin, %	85 (82-97)	89 (76-99)	90 (76-100)	81 (73-89)	.975	.110
Antithrombin, %	97 (96-104)	95 (84-106)	96 (85-106)	90 (75-102)	.226	.238
Fibrinogen, g/L	3.14 (2.71-3.39)	6.27 (5.36-7.28)	6.06 (5.26-7.17)	6.94 (5.98-8.17)	<.0001	.0059
PAI-1, ng/mL	0.60 (0.10-0.75)	2.60 (1.75-3.60)	2.50 (1.70-3.60)	3.15 (2.33-5.58)	<.0001	.054
Activation of coagulation and fibrinolysis						
D-dimer, ng/mL	290 (205-445)	1110 (690-2013)	1045 (660-1975)	1435 (908-3073)	<.0001	.105
TAT, μg/mL	2.90 (2.05-3.80)	5.35 (4.00-7.03)	5.20 (3.98-6.85)	7.10 (5.85-10.88)	<.0001	.006
PAP, ng/mL	544 (437-707)	1273 (1017-1726)	1280 (1012-1730)	1225 (1069-1700)	<.0001	.963
Thrombin generation assay						
ETP, nM IIa xmin	606 (422-773)	750 (547-906)	728 (547-892)	789 (326-1010)	.027	.860
Peak, nM IIa	167 (123-215)	198 (141-246)	203 (142-247)	186 (94-255)	.106	.430
Lag time, min	2.00 (1.67-2.00)	2.67 (2.29-3.07)	2.62 (2.28-3.00)	3.00 (2.39-3.80)	<.0001	.168
Velocity index, nM IIa/min	77 (62-112)	92 (62-120)	92 (62-122)	72 (40-113)	.647	.243
Clot lysis time, min	66 (62-70)	79 (65-91)	78 (65-90)	85 (64-129)	<.001	.213

Note: The results are presented as median (interquartile range). Comparisons between the two groups were made using the student t-test or Mann-Whitney U test, as appropriate.

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor type 1; PAP, plasmin-antiplasmin; PT, prothrombin time; TAT, thrombin-antithrombin; VWF, von Willebrand factor.

3 | RESULTS

3.1 | Patient characteristics

A total of 102 patients with COVID-19 were included in this study, of which 12 were admitted to an intermediate care unit or ICU (Table 1). Because a proportion of patients in general wards are not eligible for transfer to the ICU (due to old age or a high coexisting disease burden), and since respiratory impairment is the main indication for COVID-19 hospital care, level of respiratory support might be a better reflection of COVID-19 severity. Thus, we additionally stratified patients based on the level of respiratory support at time of blood sampling. Of note, of the 12 patients admitted to a high care unit, 9 received higher, 2 received lower, and 1 received no respiratory support. Patients with higher respiratory support received higher doses of LMWH at the time of blood sampling (Table 1). Patients with more severe disease, as reflected by admission to higher-level care units or higher respiratory support, had significantly higher CRP levels and WBC counts. Eleven patients died within 30 days of hospital admission, and all deaths were due to complications of COVID-19 (Table 1). None of the patients had objectively confirmed VTE within 30 days of hospital admission; 41 (40%) patients were screened for suspicion of VTE by computed tomography (n = 40) or lung perfusion scintigraphy (n = 1).

3.2 | Patients with COVID-19 are hypercoagulable relative to healthy controls

First, we studied the hemostatic status in patients with COVID-19 compared to healthy controls (Table 2). Patients with COVID-19 had a prolonged PT, elevated factor VIII, VWF, fibrinogen, and PAI-1 plasma levels, and decreased ADAMTS13 plasma levels in comparison to

healthy controls. Plasma prothrombin and antithrombin levels did not differ between patients and healthy controls. However, a proportion of patients had clearly decreased prothrombin and antithrombin levels; 23 (23%) patients had prothrombin levels < 75%, and 14 (14%) patients had antithrombin levels < 75%, and most of these patients (20/23 for low prothrombin and 11/14 for antithrombin) were admitted to general wards. Markers of in vivo activation of coagulation and fibrinolysis, D-dimer, TAT, and PAP all were significantly higher in patients with COVID-19 compared to controls. D-dimer and PAP levels, but not TAT levels, were associated with CRP ($R^2 = 0.11$, $P < .01$; $R^2 = 0.086$, $P < .01$; $R^2 = 0.001$, $P = .76$, respectively). In addition, thrombomodulin-modified thrombin generation was higher in patients compared to controls (despite anticoagulation). Ex vivo fibrinolytic potential was decreased in patients compared to healthy controls.

3.3 | Hemostatic profiles of patients with COVID-19 stratified by level of care

Second, we compared the hemostatic status of patients admitted to general wards with patients admitted to intermediate care units or ICUs (Table 2). PT, platelet counts, factor VIII, and VWF plasma levels were similar between general ward and high-care patients. Plasma levels of ADAMTS13 were lower in patients receiving high care in comparison to general ward patients. There was no difference in prothrombin and antithrombin levels between general ward and high-care patients. Fibrinogen and PAI-1 levels were higher in patients who received higher care, although the difference was not statistically significant ($P = .06$ and $P = .05$, respectively). Patients with COVID-19 who received a higher level of care had higher TAT levels, but D-dimer and PAP levels did not differ between the two groups. Ex vivo thrombin generation did not differ between general ward and high-care patients, although high-care patients received higher doses of LMWH (and had significantly higher anti-Xa levels; Table 1). Clot lysis time was comparable between general ward and high-care patients.

3.4 | Hemostatic profiles of patients with COVID-19 stratified by level of respiratory support

Finally, we assessed severity of disease by level of respiratory support. Patients with a higher level of respiratory support had similar PT and platelet counts, and increased factor VIII and VWF plasma levels in comparison with patients who required no or minimal respiratory support, as shown in Table 3. ADAMTS13 levels were lower in patients with respiratory support compared to patients without respiratory support. Levels of prothrombin and antithrombin did not differ between patients with different levels of respiratory support. Fibrinogen levels increased significantly with increasing respiratory support. PAI-1 levels did not differ between patients with different levels of respiratory support. Markers of in vivo activation of coagulation, D-dimer, and TAT were higher in patients with higher respiratory support, but PAP levels did not differ ($P = .051$). As for ex vivo thrombin generation, ETP, peak thrombin, and velocity index did not statistically differ based on respiratory support. However, these results are confounded by higher anti-Xa levels in these patients (Table 1). Lag times increased with increasing respiratory support and correlated with the PT ($r^2 = 0.247$, $P < .001$). Clot lysis time was longer in patients who received higher respiratory support compared to patients who received no pulmonary support.

3.5 | Hemostatic markers are associated with short-term mortality

Patients with COVID-19 who died within 30 days from hospital admission had prolonged PT, lower platelet counts, similar factor VIII plasma levels, elevated VWF, and decreased ADAMTS13 plasma levels at hospital admission in comparison to patients who were alive at 30-day follow up (Table 4). Admission plasma levels of prothrombin and antithrombin were significantly lower in patients who died compared to survivors. Fibrinogen and PAI-1 levels were comparable between survivors and nonsurvivors. Also, D-dimer, TAT, and PAP levels did not differ between COVID-19 survivors and nonsurvivors. Ex vivo thrombin generation was decreased in patients who died with corresponding increased anti-Xa levels. Clot lysis time at admission was comparable between survivors and nonsurvivors.

4 | DISCUSSION

We studied the hemostatic status of 102 patients with COVID-19 in samples taken within 7 days of hospital admission in relation to disease severity and 30-day mortality. Patients with COVID-19 with more severe disease, as reflected by increased respiratory support, had higher factor VIII, VWF, and fibrinogen plasma levels; increased in vivo activation of coagulation and fibrinolysis; and increased ex vivo clot lysis times. These findings are in accordance with previous smaller studies where severity of disease was based on the level of care.[18,19] Moreover, the results of our study demonstrate a lower platelet count, high VWF/low ADAMTS13 levels, and lower plasma levels of prothrombin and antithrombin on admission in patients who died within 30 days of hospital admission, which may suggest that a thrombotic microangiopathy with a subsequent coagulation activation and consumption of coagulation factors contributes to disease progression of COVID-19.

We confirm and extend published findings on hemostatic alterations in COVID-19 and compare hemostatic profiles between patients stratified according to level of care and according to level of respiratory support. Previous studies were either small or assessed a limited number of hemostatic tests, but in aggregate these studies are in line with our findings on changes in the VWF/ADAMTS13 axis,[18,30-33] in vivo and ex vivo activation of coagulation,[18,19,25,34-36] and in vivo and ex vivo fibrinolytic status.[18-20,26]

We first assessed disease severity according to level of care and found, in line with existing literature,[18,19] that patients receiving a higher level of care had increased plasma levels of markers of in vivo

TABLE 3 Hemostasis tests in patients with COVID-19 according to degree of respiratory support

	No respiratory support (n = 38)	Nasal cannula/mask ≤5 L O_2 (n = 46)	Higher respiratory support[a] (n = 18)
Standard hemostasis tests			
PT, s	14.9 (14.1-15.5)	15.6 (14.2-16.6)	15.9 (14.5-16.9)
Platelet count, $\times 10^9$/L	235 (149-296)	228 (178-314)	305 (180-400)
Additional hemostasis tests			
Factor VIII, %	223 (161-274)	209 (156-242)	280 (183 -299)^^
VWF, %	308 (234-435)	346 (245-416)	439 (354-481)#,^
ADAMTS13, %	76 (58-94)	66 (42-77)*	58 (42-78)#
Prothrombin, %	90 (77-99)	90 (76-100)	81 (72-96)
Antithrombin, %	99 (90-105)	94 (84-108)	92 (76-103)
Fibrinogen, g/L	5.81 (4.21-6.52)	6.55 (5.48-7.36)**	7.31 (6.04-8.43)###,^
PAI-1, ng/mL	2.90 (1.45-3.65)	2.20 (1.78-3.50)	2.95 (2.50-5.50)
Activation of coagulation and fibrinolysis			
D-dimer, ng/mL	825 (545-1563)	1245 (800-2018)*	1580 (850-3273)##
TAT, μg/mL	5.15 (3.68-6.10)	5.30 (4.15-7.00)	7.00 (4.45-10.03)#,^
PAP, ng/mL	1201 (798-1535)	1345 (1046-1898)	1422 (1229-2107)
Thrombin generation assay			
ETP, nM IIa xmin	750 (590-887)	746 (501-892)	664 (298-1064)
Peak, nM IIa	199 (160-242)	198 (126-240)	239 (139-276)
Lag time, min	2.29 (1.99-2.63)	2.95 (2.33-3.50)***	3.17 (2.66-3.96)###
Velocity index, nM IIa/min	95 (72-124)	88 (59-116)	72 (27-126)
Anti-Xa, U/mL	0.03 (0.00-0.09)	0.04 (0.01-0.10)	0.12 (0.05-0.26)###,^
Clot lysis time, min	74 (61-89)	79 (71-90)	86 (80-117)##

Note: The results are presented as median (interquartile range). Comparisons among the three groups were made using the Kruskall-Wallis test. Differences between groups were thereafter evaluated using the Mann-Whitney U test. None versus nasal cannula ≤ 5L O_2: *<0.05, **<0.01, ***<0.001; none versus higher ventilation requirements: #<0.05, ##<0.01, ###<0.001; nasal cannula ≤ 5L O_2 versus higher ventilation requirements: ^<0.05, ^^<0.01, ^^^<0.001.

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor type 1; PAP, plasmin-antiplasmin; PT, prothrombin time; TAT, thrombin-antithrombin; VWF, von Willebrand factor.

[a]Respiratory support in this group comprised > 5 L O_2 by nasal cannula/mask (n = 14), noninvasive ventilation (n = 2), and intubation (n = 2).

activation of coagulation and fibrinolysis. Ex vivo thrombin generation was higher compared to controls and similar in patients in general wards and patients in higher levels of care, despite significantly higher doses of LMWH in patients in higher levels of care, confirming our previous findings that current anticoagulant strategies might not be sufficient[18] and suggesting that patients with COVID-19 are profoundly hypercoagulable before administration of anticoagulants. However, as bleeding complications have also been associated with (severe) COVID-19,[37] increasing doses of anticoagulant therapy is not without risks, and further research on optimal anticoagulant strategies are warranted. Currently, there are numerous clinical trials being conducted on optimal anticoagulant strategies in patients with COVID-19, for example, on dosing of LMWH (NCT04367831, NCT04359277, NCT04406389) and the use of rivaroxaban as an alternative to LMWH (NCT04416048). It has been shown that heparin resistance is frequent in patients with COVID-19[38], which may indicate that other drug classes, notably direct oral anticoagulants, may be more effective. However, the concept of heparin resistance in COVID-19 has been challenged.[39]

We also demonstrated that hemostatic parameters became progressively more prothrombotic with increasing respiratory support, with the exception of prothrombin and antithrombin, platelet

TABLE 4 Hemostasis tests on admission in patients with COVID-19 who died or were alive at 30 days after hospital admission

	Survivors (30-day follow-up) (n = 92)	Nonsurvivors (30-day follow-up) (n = 10)	*P*-value
Standard hemostasis tests			
PT, s	15.1 (14.0-16.3)	16.5 (15.8-17.3)	.008
Platelet count, $\times 10^9$/L	250 (180-328)	159 (113-244)	.034
Additional hemostasis tests			
Factor VIII, %	219 (162-273)	196 (118 - 260)	.365
VWF, %	340 (247-434)	470 (309-591)	.031
ADAMTS13, %	70 (51-83)	57 (40-66)	.048
Prothrombin, %	90 (78-101)	74 (69-86)	.008
Antithrombin, %	96 (85-106)	86 (76-96)	.029
Fibrinogen, g/L	6.17 (5.33-7.31)	6.64 (5.38-7.28)	.857
PAI-1, ng/mL	2.60 (1.70-3.60)	2.85 (1.95-4.15)	.609
Activation of coagulation and fibrinolysis			
D-dimer, ng/mL	1090 (663-1968)	1330 (868-2545)	.222
TAT, μg/mL	5.30 (4.00-7.00)	5.70 (4.30-8.30)	.558
PAP, ng/mL	1278 (1017-1747)	1162 (978-1426)	.396
Thrombin generation assay			
ETP, nM IIa xmin	761 (562-903)	516 (353-783)	.057
Peak, nM IIa	212 (149-256)	138 (86-174)	.009
Lag time, min	2.62 (2.28-3.00)	3.64 (3.25-4.00)	<.001
Velocity index, nM IIa/min	96 (64-121)	60 (38-68)	.011
Anti-Xa	0.04 (0.01-0.10)	0.27 (0.10-2.67)	<.001
Clot lysis time, min	78 (64-91)	86 (77-96)	.216

Note: The results are presented as median (interquartile range). Comparisons between the two groups were made using the Mann-Whitney U test.

Abbreviations: ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor type 1; PAP, plasmin-antiplasmin; PT, prothrombin time; TAT, thrombin-antithrombin; VWF, von Willebrand factor.

count, and ex vivo thrombin generation. In addition, D-dimer, CRP, and WBC counts increased with increasing respiratory support requirements. Also, the PT of patients with COVID-19 is prolonged compared to controls irrespective of severity of disease, which may be erroneously interpreted as an increasingly "auto-anticoagulated" state. We know from various other clinical situations that a prolongation of the PT in a patient with complex changes in the hemostatic system does not necessarily indicate a decrease in hemostatic potency.[40] Indeed, the hemostatic status of patients with COVID-19 is particularly procoagulant, as evidenced by plasma markers of in vivo activation of coagulation, a VWF/ADAMTS13 unbalance, enhanced thrombin-generating capacity with high fibrinogen levels, and reduced plasma fibrinolytic potential. The prolonged PT thus should never be a contraindication for prophylactic anticoagulant therapy.

VWF plasma levels increased with increasing disease severity, which is likely related to the massive release of cytokines that has been described in severe COVID-19.[41,42] These cytokines could damage and/or activate the endothelium, resulting in VWF release into plasma. In addition, ADAMTS13 levels decrease with increasing disease severity, which may be because ADAMTS13 is consumed when processing ultra-large VWF multimers released from the endothelium. A decline in ADAMTS13 levels with high VWF levels has been previously demonstrated in other prothrombotic states, including patients with decompensated cirrhosis, chronic uremia, and acute inflammatory states and in the postoperative period.[43] Moreover, higher VWF and lower ADAMTS13 levels at admission were associated with short-term mortality in our COVID-19 cohort. The association of a VWF/ADAMTS13 imbalance with mortality has also been demonstrated in severe sepsis and acute liver failure.[44,45] The exact mechanisms by which this imbalance contributes to disease progression are incompletely understood, but we hypothesize that a platelet-mediated thrombotic microangiopathy followed by activation of coagulation and a consumptive coagulopathy might lead to intrapulmonary (and extrapulmonary) clot formation, which could lead to multiple organ failure, and ultimately death.

The mild consumption coagulopathy that we see in a proportion of patients (with 17% of patients with a PT prolongation of >3 seconds and 14% of patients with an antithrombin plasma level < 75%) is in contrast with previous reports that dismiss a consumption coagulopathy, for example, based on the observation that antithrombin levels even in the sickest patients remain normal.[15] As only three patients in our present study were admitted to the ICU and since blood samples were obtained shortly after admission, the mild consumption coagulopathy that we see in a proportion of patients could possibly have progressed during the hospital stay. There thus may be clinical value in adding plasma levels of prothrombin and antithrombin and of VWF/ADAMTS13 to the diagnostic screen in patients with COVID-19.

In contrast with results of other studies,[20] high D-dimer levels were not associated with death, although D-dimer levels did increase with increasing respiratory support. Future larger studies should identify independent predictors of outcome and should assess whether longitudinal investigations of the various biomarkers related to outcome provide added value. A recent study on longitudinal laboratory tests in patients with COVID-19 in comparison to hospitalized patients who were COVID-19 negative reported a distinctive pattern of high fibrinogen levels and low platelet counts in the early phase of disease, with decreasing fibrinogen and increasing platelet counts in a later stage of disease.[46] However, this study did not relate longitudinal patterns of hemostatic factors with mortality.

There are several limitations to this study that need to be discussed. Although we have studied a relatively large cohort of hospitalized patients with COVID-19, the number of critically ill patients was limited. There was also some variability in time between hospital admission and blood sampling, which was inevitable due to delays of RT-PCR test results and availability of personnel in the midst of the COVID-19 epidemic in Sweden. Furthermore, blood sampling was not standardized relative to time of administration of anticoagulation. Finally, although we have demonstrated a hypercoagulable state in patients with COVID-19, it is as yet unclear whether the hemostatic profile of patients with COVID-19 is unique for this disease or whether the hemostatic changes mirror that of patients with critical (respiratory) illness. One recent study has demonstrated that patients with COVID-19 are more procoagulant by rotational thromboelastography as compared to patients with non–COVID-19 pneumonia[47], but more in-depth studies are certainly indicated.

In conclusion, we demonstrate that patients with COVID-19 have increased in vivo activation of coagulation and fibrinolysis despite anticoagulant therapy and derangements in various hemostatic proteins, which were more profound proportional to severity of disease as assessed by respiratory support. Importantly, ex vivo thrombin generation was higher compared to controls, and similar in patients in general wards and patients in higher levels of care, despite significantly higher doses of LMWH in patients in higher levels of care, suggesting that current anticoagulant strategies are insufficient. Whether laboratory tests, such as thrombin generation tests or whole blood thromboelastography, may be useful in guiding anticoagulant treatment requires further study. In addition, low platelet count; low prothrombin, antithrombin, and ADAMTS13 levels; and high levels of VWF on admission were associated with 30-day mortality. Together, these results substantiate the hypothesis that hyperactivation of hemostasis might play a role in the progression of lung injury in COVID-19 by formation of intra- and extrapulmonary clots.

RELATIONSHIP DISCLOSURE

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception and design: FM, CT, and TL; patient inclusion: SH, AL, AR, and CT; data acquisition: SH and CT; laboratory analyses: JA; analysis: FM and TL; interpretation: FM, SH, JA, AL, AR, MM, NM, CT, and TL; supervision: CT and TL; drafting of manuscript: FM and TL; and revision of the manuscript: SH, JA, AL, AR, MM, NM, and CT.

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