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Published in:
Journal of Thrombosis and Haemostasis

DOI:
10.1111/jth.15043

Publication date:
2020

Received: 11 June 2020 | Accepted: 3 August 2020

DOI: 10.1111/jth.15043

BRIEF REPORT

In vitro hypercoagulability and ongoing in vivo activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation

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Abstract

Background: COVID-19 is associated with a substantial risk of venous thrombotic events, even in the presence of adequate thromboprophylactic therapy.

Objectives: We aimed to better characterize the hypercoagulable state of COVID-19 patients in patients receiving anticoagulant therapy.

Methods: We took plasma samples of 23 patients with COVID-19 who were on prophylactic or intensified anticoagulant therapy. Twenty healthy volunteers were included to establish reference ranges.

Results: COVID-19 patients had a mildly prolonged prothrombin time, high von Willebrand factor levels and low ADAMTS13 activity. Most rotational thromboelastometry parameters were normal, with a hypercoagulable maximum clot firmness in part of the patients. Despite detectable anti-activated factor X activity in the majority of patients, ex vivo thrombin generation was normal, and in vivo thrombin generation elevated as evidenced by elevated levels of thrombin-antithrombin complexes and D-dimers. Plasma levels of activated factor VII were lower in patients, and levels of the platelet activation marker soluble CD40 ligand were similar in patients and controls. Plasmin-antiplasmin complex levels were also increased in patients despite an in vitro hypofibrinolytic profile.

Conclusions: COVID-19 patients are characterized by normal in vitro thrombin generation and enhanced clot formation and decreased fibrinolytic potential despite the presence of heparin in the sample. Anticoagulated COVID-19 patients have persistent in vivo activation of coagulation and fibrinolysis, but no evidence of excessive platelet activation. Ongoing activation of coagulation despite normal to intensified anticoagulant therapy indicates studies on alternative antithrombotic strategies are urgently required.

Manuscript handled by: Katsue Suzuki-Inoue

Final decision: Katsue Suzuki-Inoue, 3 August 2020

KEYWORDS
anticoagulation, coagulation, COVID-19, fibrinolysis

1 | INTRODUCTION

Patients with COVID-19 have a profound risk for venous thrombotic events. Particularly in patients admitted to an intensive care unit (ICU), rates of deep vein thrombosis and pulmonary embolism are exceedingly high, even in the presence of pharmacological thromboprophylaxis.[1,2] In addition to macrovascular thrombotic events, microvascular thrombosis has been proposed to contribute to disease progression, with pulmonary clots contributing to respiratory failure,[3-5] and clots in other vascular beds to multiple organ failure.[6,7] Anticoagulant treatment has been shown to reduce mortality, perhaps because of reduction of microvascular thromboses.[8]

The high thrombosis risk in COVID-19 patients has been linked to a hypercoagulable state that has not been well defined. The in vivo hyperactivation of coagulation appears to be linked to a massive inflammatory response coupled with increases in acute phase proteins including fibrinogen,[9] and involvement of neutrophil extracellular traps (NETs),[10] which are newly recognized actors in thrombosis. Routine hemostasis tests show mild prolongations in prothrombin time and activated partial thromboplastin time, and mild thrombocytopenia in some patients, but massively elevated levels of D-dimer in many patients,[9] that appear to have prognostic value.[11] Whole blood thromboelastography has demonstrated a hypercoagulable profile,[12,13] and one of these studies concluded that the COVID-19 coagulopathy does not have elements of typical disseminated intravascular coagulation, as has been suggested by others.[13] Notably, increased (major) bleeding complications have been described in COVID-19 patients, especially in the critically ill, suggesting a fragile balance in hemostatic status of these patients,[14] although others have demonstrated bleeding risks comparable to patients with non-COVID-19 acute respiratory syndromes.

In a large academic medical center in Barcelona, Spain, to which approximately 600 patients were admitted at the peak of the COVID-19 pandemic, the initial reports on thrombosis and hypercoagulability and their own observations led to an intensified thromboprophylactic regimen for part of the admitted COVID-19 patients, particularly those with more advanced disease. Although an anticoagulant protocol was instituted, during the period of our study, this protocol was poorly adhered to and individualized decisions on anticoagulant dosing were taken. We aimed to study the effects of this individualized anticoagulant therapy on the hemostatic status of these patients.

Essentials

- COVID-19 patients are at increased risk for venous thrombotic events, despite thromboprophylaxis.
- Hemostatic status of 23 COVID-19 patients on anticoagulant therapy was studied in plasma.
- In vitro: normal coagulation, enhanced clot formation and hypofibrinolysis despite heparin.
- Persistent in vivo activation of coagulation and fibrinolysis despite anticoagulant therapy.

2 | MATERIALS AND METHODS

2.1 | Patients

We included 23 patients that were admitted with COVID-19 (which was confirmed by PCR) to Hospital Clínic Barcelona, Spain, in April 2020. Almost all patients received the low molecular weight heparin (LMWH) enoxaparin. Ethical approval from the Medical Ethical Committee Hospital Clínic Barcelona (2020/0371) was obtained. All patients, or in the case of incapacity their consultee, gave informed consent or assent, respectively, for participation in this study. Twenty healthy controls were included to establish reference values for the various assays performed. Exclusion criteria for healthy controls were age younger 18 years, pregnancy, hereditary thrombophilia or hemophilia, use of anticoagulant medications, history of venous thromboembolic events, and blood (product) transfusion up to 7 days before inclusion.

2.2 | Sampling

Citrated blood samples were taken 4 (2-6) days after admission to the hospital (9 [6-13] days after onset of symptoms) on either a general ward or ICU by venipuncture or from dedicated arterial lines. In all patients, anticoagulation was started on admission. Blood samples were either used immediately for rotational thromboelastometry (ROTEM) measurements or processed to platelet-poor plasma within 30 minutes of the blood draw by double centrifugation at 2500*g* for 15 minutes, and subsequently stored at −80°C until used for analyses. Complete blood cell counts, and creatinine, total bilirubin, and C-reactive protein were measured as part of routine clinical care by the Centre de Diagnòstic Biomèdic at the Hospital Clínic Barcelona.

2.3 | Assays

We measured PT, activated partial thromboplastin time, international normalized ratio, prothrombin, antithrombin, fibrinogen, and D-dimer on an automated coagulation analyzer (STACompact 3, Stago) using reagents and protocols from the manufacturer. Von Willebrand factor (VWF) plasma levels were determined with an in-house ELISA using commercially available polyclonal antibodies against VWF (DAKO). Plasma

activity of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) was measured using the FRETS-VWF73 assay (Peptanova). Levels of VWF and ADAMTS13 in pooled normal plasma were set at 100%, and values obtained in test plasmas were expressed as a percentage of pooled normal plasma. Plasminogen activator inhibitor type 1 levels were quantified by commercially available ELISA from R&D Systems. Cell-free DNA was quantified using the Quant-iT PicoGreen dsDNA assay kit (Fisher Scientific), as described previously.[16] The concentration of myeloperoxidase DNA complexes in plasma was determined by ELISA, as previously described.[17]

ROTEM analyses were performed on a ROTEM sigma according to the manufacturers' instructions. Thrombin generation was performed as previously described[18] using commercially available reagents containing recombinant tissue factor (final concentration: 5 pmol/L), phospholipids (final concentration: 4 μmol/L), and soluble thrombomodulin (the concentration of which is not revealed by the manufacturer) (Thrombinoscope BV). Anti-activated factor X (anti-Xa) levels were measured on an automated analyzer (ACL 300 TOP) using Heparin LRT (Hyphen Biomed).

Plasma fibrinolytic potential was estimated by studying lysis of a tissue factor-induced clot by exogenous tissue plasminogen activator by monitoring changes in turbidity during clot formation and subsequent lysis, as described previously.[19] Samples that were still clotted at 3 hours after the start of the experiment were arbitrarily assigned a clot lysis time (CLT) of 180 minutes.

Plasma levels of thrombin-antithrombin (TAT) complexes, prothrombin fragment 1 + 2 (F1 + 2), plasmin-antiplasmin (PAP) complexes, and soluble CD40 ligand were quantified by commercially available ELISAs (Siemens, for TAT and F1 + 2, Technoclone, for PAP, and R&D Systems, Bio-techne, for sCD40L). Plasma levels of activated factor VII were quantified using the Hemoclot factor VIIa kit (Hyphen Biomed).

2.4 | Statistical analyses

Statistical analyses were performed using GraphPad Prism, version 8.3.1 (San Diego, CA). The results were presented as numbers (percentages) for categorical variables and medians [interquartile ranges] for continuous variables. Test results were compared between COVID-19 patients and healthy controls, and between patients admitted to the ICU and the ward, using the Mann-Whitney *U* test. Relations between laboratory parameters were made by simple linear regression. *P* values < .05 were considered statistically significant.

3 | RESULTS AND DISCUSSION

3.1 | Abnormalities in diagnostic hemostasis tests in COVID-19 patients

We studied 23 patients of whom 12 were admitted to the ICU, with 11 on general wards. None of the ward patients later had to be admitted to the ICU. Three ICU patients developed thrombotic complications: one pulmonary embolism, one myocardial infarction, and one distal ischemia of the fingers. General patient characteristics are shown in Table 1. Most ICU patients received higher anticoagulant doses compared with ward patients; Table 1). Routine diagnostic hemostasis tests and plasma levels of markers for NETs are shown in Table 2. Compared with healthy controls, patients had a prolonged prothrombin time and international normalized ratio, which are largely explained by decreased FVII levels that strongly correlated with the prothrombin time ($r^2 = .56$, $P < .0001$). In addition, patients had a decreased platelet count, elevated fibrinogen levels, slightly decreased levels of prothrombin and antithrombin, high levels of VWF and FVIII, and low levels of ADAMTS13. Of note, four patients had prothrombin and antithrombin levels < 25%, and two other patients had ADAMTS13 levels < 10%. These results are consistent with a mild consumption coagulopathy with a thrombogenic VWF profile. Plasma levels of plasminogen activator inhibitor type 1 were approximately 3.7 times higher in COVID-19 patients compared with controls. Markers of NETs were modestly elevated in patients compared with controls, and did not differ between patients that were or were not admitted to ICU, which may argue against a key role of NETs in the pathogenesis of COVID-19 associated sequelae, as was suggested previously. Indeed, unlike previously described,[10] NET markers in our cohort did not correlate with C-reactive protein, D-dimer, platelet count, or use of mechanical ventilation.

3.2 | Normal to hypercoagulable ex vivo clot formation, thrombin generation, and mild hypofibrinolysis

We next studied hemostatic potential of COVID-19 patients by three global tests (Table 3). ROTEM parameters were largely within the normal range, except for elevated maximum clot firmness in extem, intem, and fibtem in 6, 8, and 11 patients, respectively. A limitation of these analyses was that normal ranges were not locally established, but taken from the ROTEM user manual. Of note, ROTEM extem and fibtem reagents contain polybrene, which neutralizes heparin present in many of these samples. Thrombomodulin-modified thrombin generation was preserved on a group level, but individual patients were clearly hyper- or hypocoagulable. The patient samples that generated little thrombin contained high levels of LMWH as evidenced by anti-Xa activity assays, whereas hypercoagulable samples generally had low to undetectable anti-Xa activity, although one patient had marked thrombin generation even in the presence of high anti-Xa levels. ETP and peak thrombin levels were inversely correlated with anti-Xa levels ($r^2 = .16$, $P = .055$ and $r^2 = .20$, $P = .03$; without one clear outlier, these correlations became much stronger $r^2 = .54$, $P < .0001$ and $r^2 = .50$, $P = .0002$). Samples taken from patients admitted to the ICU generated substantially less thrombin, but this was directly related to much higher anti-Xa levels in samples taken from patients on the ICU compared with ward patients

TABLE 1 Patient characteristics

Variable	COVID-19 Patients (n = 23)	COVID-19 Patients ICU (n = 12)	COVID-19 Patients Ward (n = 11)	Healthy Controls (n = 20)	*P* Value Patients vs Controls	*P* Value ICU vs Ward
Age	64 [53-74]	69 [57-76]	58 [42-74]	44 [39-50]	<.001	.267
Female	9 (39%)	6 (50%)	3 (27%)	9 (45%)	.697	.265
Body mass index (kg/m^2)	30 [27-34]	32 [27-35]	29 [27-31]	24 [20-27]	<.0001	.285
SOFA score	3.0 [1.0-6.0]	5.5 [3.3-7.8]	2.0 [0.0-3.0]			.01
APACHE score	12.0 [5.0-16.0]	15.5 [12.0-17.8]	5.0 [3.0-10.0]			<.0001
Pulmonary support						
Intubated	12 (52%)	12 (100%)	0 (0%)			<.0001
Noninvasive ventilation	1 (4%)	0 (0%)	1 (9%)			.286
Nasal oxygen	10 (43%)	0 (0%)	10 (91%)			<.0001
PaO_2/FiO_2 (PaFI)	367 [208-420]	239 [130-414]	418 [325-420]			.121
Respiratory rate (/min)	22 [18-28]	27 [26-32]	18 [18-20]			.0002
SIRS criteria (≥2)	8 (35%)	6 (50%)	2 (18%)			.110
DIC score (≥5)	2 (9%)	1 (8%)	1 (9%)			.949
Glasgow coma scale (≤8)	3 (13%)	2 (17%)	1 (9%)			.590
Underlying (chronic) disease						
Cardiovascular disease	13 (57%)	8 (67%)	5 (45%)			.305
Cirrhosis	5 (22%)	1 (8%)	4 (36%)			.104
Diabetes	4 (17%)	2 (17%)	2 (18%)			.924
Onset of symptoms before admission (days)	6 [5-8]	7 [5-10]	6 [5-7]			.626
Blood sampling day (since admission)	4 [2-6]	5 [4-7]	2 [1-2]			<.0001
Baseline laboratory values						
Creatinine (mg/dL)	0.80 [0.42-2.10]	0.63 [0.29-2.09]	0.80 [0.56-2.10]			.281
C-reactive protein (mg/L)	2.33 [0.70-5.57]	0.77 [0.42-2.59]	3.28 [2.33-8.96]			.009
Lactate (mg/dL)	15 [12-16]	15 [13-19]	15 [9-15]			.374
LDH (U/L)	307 [236-424]	345 [258-660]	305 [193-354]			.300
pH	7.41 [7.33-7.45]	7.45 [7.32-7.47]	7.38 [7.33-7.43]			.169
Total bilirubin (mg/dL)	0.80 [0.40-1.30]	0.80 [0.45-1.23]	0.60 [0.40-1.30]			.682
WBC count ($\times 10^9/L$)	6.3 [3.4-11.8]	10.5 [6.7-15.3]	3.5 [3.1-4.5]	5.88 [5.26-7.93]	.995	.007
Anticoagulation (LMWH)						
No	2 (9%)	0 (0%)	2 (18%)			.122
<0.5 mg/kg/d	7 (30%)	2 (17%)	5 (45%)[a]			.134
0.5-1.5 mg/kg/d	9 (39%)	6 (50%)	3 (27%)			.265
≥1.5 mg/kg/d	5 (22%)	4 (33%)[b]	1 (9%)			.159

(Continues)

TABLE 1 (Continued)

Variable	COVID-19 Patients (n = 23)	COVID-19 Patients ICU (n = 12)	COVID-19 Patients Ward (n = 11)	Healthy Controls (n = 20)	*P* Value Patients vs Controls	*P* Value ICU vs Ward
Outcome						
Duration of hospital stay in days (n = 21)	25 [7.5-44]	43 [21-46]	11 [5.0-26]			.015
Still in hospital	2 (9%)	2 (17%)	0 (0%)			.157
Death	4 (17%)	2 (17%)	2 (18%)			.924

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 chi-squared test, as appropriate.

Abbreviations: DIC, disseminated intravascular coagulation; ICU, intensive care unit; LDH, lactate dehydrogenase; LMWH, low-molecular-weight heparin; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment; WBC, white blood cell.

[a]One patient received tinzaparin sodium 3500 International Units per day.

[b]One ICU patient received intravenous infusion of unfractionated heparin.

TABLE 2 Various laboratory values in healthy controls and COVID patients with additional subdivision in ICU and non-ICU patients

	Controls (n = 20)	COVID-19 Patients (n = 23)	*P* Value	ICU (n = 12)	Non-ICU (n = 11)	*P* Value
Standard hemostasis tests						
PT (s)	10.9 [10.7-11.3]	15.9 [15.3-18.6]	<.0001	15.6 [15.2-16.4]	18.6 [15.8-19.8]	.046
APTT (s)	33.4 [31.4-35.0]	34.6 [31.2-39.7]	.113	33.7 [31.2-41.5]	36.0 [31.2-39.7]	.707
INR	1.01 [0.99-1.05]	1.15 [1.11-1.34]	<.0001	1.13 [1.10-1.19]	1.34 [1.15-1.42]	.049
Platelet count (10^9/L)	244 [184-323]	167 [136-250]	.039	196 [127-293]	167 [154-239]	.940
Additional hemostasis tests						
Fibrinogen (g/L)	3.00 [2.77-3.50]	4.51 [2.82-5.15]	.021	3.93 [3.00-4.88]	5.02 [1.72-5.52]	.413
Prothrombin (%)	130 [113-144]	85 [50-99]	<.0001	84 [64-96]	87 [48-100]	.893
Antithrombin (%)	113 [104-126]	102 [51-121]	.016	106 [76-126]	85 [43-115]	.206
VWF (%)	132 [95.5-176]	306 [200-421]	<.0001	367 [296-464]	216 [180-319]	.015
FVIII (%)[a]	88 [72-110]	161 [129-216]	<.0001	172 [136-235]	160 [96-195]	.234
FVII (%)[a]	90 [81-99]	71 [55-85]	.0034	76 [66-91]	60 [44-78]	.052
ADAMTS13 (%)	101 [83.3-116]	47.3 [25.8-66.1]	<.0001	46.2 [27.4-57.8]	59.9 [25.8-70.3]	.449
PAI-1 (ng/mL)	0.70 [0.33-1.45]	2.60 [2.00-3.08]	<.0001	2.35 [1.70-3.08]	2.60 [2.00-5.30]	.705
NET markers						
Cell-free DNA (μg/mL)	0.89 [0.87-0.96]	1.28 [1.15-1.45]	<.0001	1.33 [1.27-1.47]	1.22 [1.12-1.37]	.062
MPO-DNA complexes (AU)	0.07 [0.05-0.10]	0.14 [0.10-0.57]	<.001	0.13 [0.09-0.17]	0.18 [0.12-0.79]	.094

Note: The results are presented as median [interquartile range]. Comparisons between the two groups were made using the Mann-Whitney *U* test for nonparametric data.

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; APTT, activated partial thromboplastin time; ICU, intensive care unit; INR, international normalized ratio; MPO, myeloperoxidase; NET, neutrophil extracellular trap; PAI-1, plasminogen activator inhibitor type 1; PT, prothrombin time; VWF, von Willebrand factor.

[a]Missing data of one (non-ICU) patient for FVII and FVIII analyses (n = 22).

(Table 3). Because samples were not taken at specific time points relative to the last LMWH injection, and because there was a substantial difference in dosing and timing of LMWH administration between ICU and ward, this likely explains the difference in anti-Xa and thrombin generating capacity between ICU and ward patients.

Plasma CLT was higher in COVID-19 patients compared to healthy controls, but similar between patients on ICU and ward. Five patients had substantially elevated CLT (>100 minutes). Two of these had underlying liver disease and may have been hypofibrinolytic related to decompensating liver disease as we have described previously[20]; the other three were all admitted to the

TABLE 3 Global hemostatic tests

	Controls (n = 20)	COVID-19 Patients (n = 23)	*P* Value	ICU (n = 12)	Non-ICU (n = 11)	*P* Value
ROTEM[a]	Reference ranges					
CT Ex (s)	50-80	70.0 [65.5-72.8]		70.5 [66.3-75.0]	69.5 [64.0-71.0]	.352
MCF Ex (mm)	55-72	68.0 [64.5-74.3]		71.0 [67.0-75.8]	66.5 [61.8-69.5]	.154
LY60 Ex (%)	94-100	99.5 [96.0-100]		100 [100-99.3]	96.0 [94.5-97.8]	<.001
CT Int (s)	161-204	180 [164-204]		193 [179-228]	170 [157-178]	.013
MCF Int (mm)	51-69	64.5 [60.8-71.3]		68.0 [63.3-71.8]	62.0 [57.0-68.5]	.261
MCF Fibtem (mm)	6-21	21.5 [13.8-26.0]		20.5 [13.3-23.0]	24.5 [17.5-29.0]	.144
TGA						
ETP (nmol/L IIa × min)	385 [243-515]	472 [153-807]	.328	185 [7.75-459]	789 [663-841]	.002
Peak (nmol/L IIa)	77.5 [47.3-126]	100 [25.0-176]	.348	40.0 [1.89-95.8]	168 [145-186]	.008
Lag time (min)	2.17 [1.67-2.33]	2.33 [1.67-3.00]	.236	2.33 [1.59-3.15]	2.33 [1.67-3.00]	.682
Velocity index (nmol/L IIa/ min)	31.0 [15.8-62.8]	54.0 [19.0-82.0]	.265	26.0 [1.50-62.8]	74.0 [54.0-88.0]	.022
Anti-Xa (U/mL)		0.13 [0.03-0.61]		0.44 [0.18-0.65]	0.03 [0.00-0.12]	.008
CLT (min)	68.1 [59.8-71.7]	80.8 [69.5-94.3]	.007	83.8 [70.6-99.4]	74.7 [61.2-89.9]	.449

Note: The results are presented as median [interquartile range]. Comparisons between the two groups were made using the Mann-Whitney *U* test for nonparametric data.

Abbreviations: anti-Xa, anti-activated factor X; CLT, clot lysis time; CT, clot time; ETP, endogenous thrombin potential; Ex, extrinsic TEM; ICU, intensive care unit; In, intrinsic TEM; LY60% of maximum MCF at 60 minutes; MCF, maximal clot firmness; ROTEM, rotational thromboelastometry; TGA, thrombin generation assay.

[a]Missing data of one (non-ICU) patient for ROTEM analyses (n = 22).

TABLE 4 Markers of in vivo activation of coagulation, fibrinolysis, and platelets

	Controls (n = 20)	COVID-19 Patients (n = 23)	*P* Value	ICU (n = 12)	Non-ICU (n = 11)	*P* Value
D-dimer[a] (ng/mL)	208 [157-309]	1110 [573-5305]	<.0001	2535 [860-7848]	565 [425-2188]	.025
TAT (μg/mL)	1.55 [1.40-2.20]	7.30 [4.50-12.2]	<.0001	7.15 [4.45-8.78]	11.8 [4.50-24.1]	.355
F1 + 2 (pmol/L)	206 [158-269]	191 [145-314]	.966	218 [140-449]	186 [145-314]	.964
VIIa[a] (mU/mL)	52.0 [41.8-63.6]	27.2 [21.7-41.7]	.002	32.2 [24.5-45.2]	25.5 [10.2-30.1]	.069
PAP (ng/mL)	193 [170-240]	984 [648-2377]	<.0001	862 [484-2324]	1017 [730-2590]	.309
sCD40L (ng/mL)	52.0 [16.5-129]	83.0 [33.0-177]	.465	52.0 [20.0-148]	130 [63.0-183]	.217

Note: The results are presented as median [interquartile range]. Comparisons between the two groups were made using the Mann-Whitney *U* test for nonparametric data.

Abbreviations: F1 + 2, prothrombin fragment F1 + 2; ICU, intensive care unit; PAP, plasmin-antiplasmin; sCD40L, soluble CD40 Ligand; TAT, thrombin-antithrombin; VII(a), (activated) blood coagulation factor VII.

[a]Missing data of one (non-ICU) patient for D-dimer and VIIa.

ICU, and hypofibrinolysis is a common feature of patients that are critically ill. Viscoelastic tests have shown evidence of fibrinolytic shutdown in one-quarter of COVID-19 patients and fibrinolytic shutdown was associated with thrombosis.[21] However, the definition of hypofibrinolysis using viscoelastic tests is not straightforward because "no lysis" is in fact part of the reference range.[22] We found "no lysis" (defined as CLT > 180 minutes) in only three patients (13%), whereas our plasma-based test detects "no lysis" in a much larger proportion of other patient populations (notably postsurgery[23] and patients with acute liver failure [73.5%][24]). This suggests that a true fibrinolytic shutdown is rare in COVID-19, which is also evidenced by highly elevated D-dimer and plasmin-antiplasmin complexes (see the following section). Importantly, LMWH decreases CLT across physiologically relevant concentrations,[25] which may be why plasma clot lysis was only mildly impaired in our patients.

Thus, COVID-19 patients have somewhat elevated clot formation, likely related to hyperfibrinogenemia, normal thrombin generating capacity despite the presence of LMWH, and hypofibrinolysis despite the presence of LMWH.

3.3 | Ongoing in vivo activation of coagulation and fibrinolysis despite low therapeutic anticoagulation in COVID-19 patients

In vivo markers of activation of coagulation are shown in Table 4. TAT and PAP complex levels are strongly elevated in patients with COVID-19, indicating ongoing thrombin and plasmin generation in COVID-19 patients despite anticoagulation with LMWH. Interestingly, TAT and PAP levels were not different between patients admitted to the ICU or to the general ward, whereas D-dimer levels were substantially higher in ICU compared with ward patients. This may indicate that ICU patients have a higher clot burden, which may be primarily intrapulmonary clots,[26,27] despite similar procoagulant activity. This might be explained by decreased (local) anticoagulant capacity in ICU patients, perhaps related to increased endothelial injury that decreases availability of thrombomodulin and endogenous heparinoids. Surprisingly, F1 + 2 levels were not increased in COVID-19 patients compared with controls, and we have no explanation for the discrepancy between TAT and F1 + 2 levels. D-dimer and TAT levels were not correlated to anti-Xa levels, but F1 + 2 levels were inversely correlated with anti-Xa levels (r^2 = .22, P = .03). The VIIa levels were lower in patients, which may point to consumption of VIIa similar to what we have previously observed in patients during the acute phase of deep vein thrombosis.[28] Indeed, VIIa levels strongly positively correlated with zymogen VII levels (r^2 = .59, P = <.0001). Soluble CD40 ligand levels were similar between patients and controls, suggesting COVID-19 patients are not characterized by excessive platelet activation.

Taken together, our data confirm a hypercoagulable status of enhanced thrombin generating capacity, enhanced ex vivo clot formation likely related to hyperfibrinogenemia, and a decreased ex vivo fibrinolytic capacity in patients with COVID-19. Interestingly, despite normal to intensified anticoagulant treatment, in vivo activation of coagulation and fibrinolysis was evident and independent of anti-Xa levels, whereas in vitro activation of coagulation proportionally decreased as a function of anti-Xa levels. Our observations that the hypercoagulable profile is more pronounced in the sicker patients are in line with the hypothesis that activation of coagulation, particularly in the pulmonary circulation, is a key feature of COVID-19 and may contribute to progression of disease.[26] These data suggest that low therapeutic anticoagulant regimens are often insufficient to downregulate coagulation activation in COVID-19 patients, and call for assessment of alternative or intensified antithrombotic strategies. However, careful individual patient assessment (especially in the critically ill) is warranted, given the increased bleeding risk that is associated with COVID-19.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Annabel Blasi: conception and design, patient inclusion, data acquisition, interpretation, revision of the manuscript; Fien A. von Meijenfeldt: interpretation, analysis, drafting of manuscript; Jelle Adelmeijer: laboratory analyses, interpretation, revision of the manuscript; Andrea Calvo: patient inclusion, interpretation, revision of the manuscript; Cristina Ibañez: patient inclusion, interpretation, revision of the manuscript; Juan Perdomo: patient inclusion, interpretation, revision of the manuscript; Juan C. Reverter: interpretation, revision of the manuscript; Ton Lisman: conception and design, analysis, supervision, interpretation, drafting of manuscript.

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How to cite this article: Blasi A, von Meijenfeldt FA, Adelmeijer J, et al. In vitro hypercoagulability and ongoing in vivo activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation. *J Thromb Haemost*. 2020;00:1–8. https://doi.org/10.1111/jth.15043