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Severe SARS-CoV-2 Infection Inhibits Fibrinolysis Leading to Changes in Viscoelastic Properties of Blood Clot: A Descriptive Study of Fibrinolysis in COVID-19

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Abstract

Background Accumulating evidence indicates toward an association between severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and procoagulatory state in blood. Thromboelastographic investigations are useful point-of-care devices to assess coagulation and fibrinolysis.

Objectives We investigated the hypothesis that the procoagulatory state in COVID-19 patients is associated with impaired fibrinolysis system.

Methods Altogether, 29 COVID-19 patients admitted to normal wards or to the intensive care unit (ICU) were included in this descriptive study. Whole blood samples were investigated by thromboelastography to assess coagulation and fibrinolysis. Additionally, standard routine coagulation testing and immunoassays for factors of fibrinolysis as plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA), plasminogen activity and α2-antiplasmin (A2AP) were performed.

Results A significantly increased lysis resistance and a significantly longer time of lysis after adding tissue plasminogen activator were observed in blood samples from ICU COVID-19 patients compared with healthy controls (maximal lysis: 3.25 ± 0.56 vs. $6.20 \pm 0.89\%$, $p = 0.0127$; lysis time: 365.7 ± 44.6 vs. 193.2 ± 16.3 seconds, $p = 0.0014$). PAI-1 activity was significantly higher in plasma samples of ICU COVID-19 patients (PAI-1: 4.92 ± 0.91 vs. 1.28 ± 0.33 U/mL, $p = 0.001$). A positive correlation

Keywords
- blood coagulation
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between the activity of PAI-1 and lysis time of the formed clot ($r = 0.70$, $p = 0.0006$) was observed.

Conclusion Our data suggest that severe SARS-CoV-2 infection is associated with impaired fibrinolytic activity in blood, where fibrinolytic inhibitors are elevated leading to an increased resistance to clot lysis. Thromboelastography could offer a tool to investigate the contribution of the fibrinolytic status to the procoagulatory condition in COVID-19.

Introduction

There is a growing evidence suggesting an association between 2019 novel coronavirus (2019-nCoV) pneumonia and significant dysregulation of the coagulation system.[1] The pathophysiology of COVID-19-associated coagulopathy seems to be complex and multifactorial, involving interplay between cellular and plasmatic elements of the haemostatic system and components of the innate immune response to the infecting pathogen.[2] Recent data suggest that a combination of activation events initiated by exposure of endothelium, platelets and leukocytes to pathogen- and damage-associated molecular patterns might be responsible for the uncontrolled stimulation of the coagulation system in COVID-19 patients. Most reports on COVID-19-associated coagulopathy focused on the increased activation of the plasmatic coagulation system.

Viscoelastic tests are whole blood analyses that have the advantage of providing information related to the cumulative effects of plasma clotting factors, platelets, leukocytes and red cells during all stages of the coagulation and fibrinolytic processes. Whereas viscoelastic coagulation devices such as thromboelastography (TEG) or rotational thromboelastometry (ROTEM) have been widely used in many fields of intensive care to guide haemostatic treatment in patients with bleeding, little is known about their use to monitor patients with disposition to thromboembolic complications.[3] A recent study showed that viscoelastic testing might be a better indicator of the postinjury hypercoagulable state than routinely used coagulation tests.[4] In addition, it has been shown that the postoperative risk for venous thromboembolism (VTE) could be predicted in patients with and without cancer by TEG[5,6] as well as in patients with major orthopaedic trauma.[7]

Recent studies described the hypofibrinolysis in COVID-19 patients.[8] The severity of illness and outcome seem to correlate with diverse coagulation parameters.[9] So high levels of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) have enhanced spontaneous fibrinolysis and were associated with worse outcomes.[10,11] The resistance to clot lysis was described by an elevated lysis index at 30 minutes in modified ROTEM[12] or a decreased clot lysis in extrinsic rotational thromboelastometry (EXTEM) and fibrinogen rotational thromboelastometry (FIBTEM).[13] Hypercoagulability was demonstrated by an increased maximum clot firmness.[12,13] Recently, a new test system (ClotPro) has been introduced as the first thromboelastographic device to investigate clot resistance to fibrinolysis.

After observing that blood samples from COVID-19 patients show different viscoelastic properties compared with healthy donors,[14,15] in this study, we systematically analyze the viscoelastic changes in the clotting system with a focus on plasmin resistance.

Methods

Study Design

Consecutive patients with COVID-19 infection were included in this retrospective observational descriptive study. All patients were diagnosed for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection by real-time polymerase chain reaction (PCR) using nasal swabs. Citrated blood samples were collected and viscoelastic investigations were initiated. Blood samples from healthy donors from the blood donation centre of Tübingen were used as controls. Medical records were independently reviewed by two physicians.

Coagulation Parameters

The following coagulation parameters were measured by the Sysmex CS-5100 system (Siemens, Marburg, Germany) with reagents from Siemens Healthcare Systems: activated partial thromboplastin time (aPTT; Dade Actin), prothrombin time (PT), international normalized ratio (INR; Dade Innovin) and D-dimer (INNOVANCE). Platelet count (PLT) was determined using Sysmex XN 9000 (Sysmex, Norderstedt, Germany).

Thromboelastographic Assays

Citrated blood samples were collected and investigated within 2 hours using a viscoelastic test system (ClotPro Enicor GmbH, Munich, Germany). In this new method, blood coagulation is determined by elastic motion TEG. ClotPro testing is initiated by pipetting citrated whole blood by an electronic pipette using an active tip containing the activator reagents. The activator reagents are mixed with blood during the pipetting process. The activated blood is investigated in a cylindrical cup. A fixed pin is inserted and the coagulation assessment is measured by rotating the cup alternately clockwise and anticlockwise while the pin remains stationary. The movement of the cup is recorded and data are transformed into thromboelastographic amplitude values plotted over time. The cup rotation is increasingly resisted and reduced depending on elastic properties of the formed clot.[16,17]

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The extrinsic assay (Ex test) and the tissue plasminogen activator assay (TPA test) were employed according to the manufacturer's instructions. Briefly, in the Ex test, clotting is initiated using tissue factor. This test seems to be sensitive for anticoagulation, fibrinogen, factor XIII and hyperfibrinolysis. The TPA test is similar to the EX test but contains 650 to 700 ng/mL additional recombinant tPA (r-tPA), an activator of plasmin, to assess the fibrinolysis resistance. The following parameter were estimated during the study: clotting time (CT), clot formation time (CFT), α angle, maximal clot firmness (MCF), lysis time (LT, time from the beginning of the clot formation until 50% of clot lysis) and maximum lysis (ML).

Plasma Factors of the Fibrinolysis System

Platelet-poor plasma (PPP) samples were separated within 2 hours after blood collection (2,500 g, 20 minutes) and stored at –80°C until further testing. PPPs were tested in batches using commercially available enzyme immunoassay (EIA) kits according to the manufacturer's instructions to determine the plasma levels of PAI-1 (Berichrom PAI, Siemens), plasminogen (Berichrom Plasminogen, Siemens), α2-antiplasmin (A2AP, Biophen α2 Antiplasmin, Hyphen Biomed, Neuville-sur-Oise, France), functional fibrinogen (Multifibern U, Siemens), immunological fibrinogen (Liaphen Fibrinogen, Hyphen Biomed) and tPA (Technozym tPA Antigen, Diapharma, West Chester, Ohio, United States). Frozen plasma samples from 11 healthy donors were used as control.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to any study-related procedure. The study protocol was approved by the institutional review board of the University of Tübingen.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.0 (La Jolla, California, United States). Unpaired *t*-test was used to analyze normally distributed results. Nonparametric tests were used when data failed to follow a normal distribution as assessed by D'Agostino and Pearson omnibus normality test. All data are provided as mean ± SEM. A *p*-value of <0.05 was assumed to represent statistical significance.

Results

Study Cohort and Clinical Manifestations

Blood samples were collected from 29 consecutive patients with PCR-confirmed COVID-19 infection who were admitted to our hospital (►**Fig. 1**). Of these, 20 (69%) patients required admission to the ICU and 22 patients (76%) were males. The patients had a median age of 59 years (interquartile range [IQR]: 40–73 years; range: 7–93 years; ►**Table 1**). Blood samples were also collected from 10 healthy donors (5 women [45%]; age range: 22–58 years).

Moderate COVID-19

Nine patients with moderate symptoms of COVID-19 were treated on normal ward. The median age of these patients was 44 years (range: 7–87 years). On the day of thromboelastographic testing, patients were hospitalized for a mean of 9 days (range: 3–18 days). Two of the nine non-ICU patients died within 30 days after viscoelastic testing. All patients received low-molecular-weight heparin (LMWH; enoxaparin 4,000 U/d).

Severe COVID-19

On the day of thromboelastographic assessment, ICU COVID-19 patients ($n = 20$) had a median Sequential Organ Failure Assessment (SOFA) score of 7 (IQR: 6–11; range: 3–15). Three

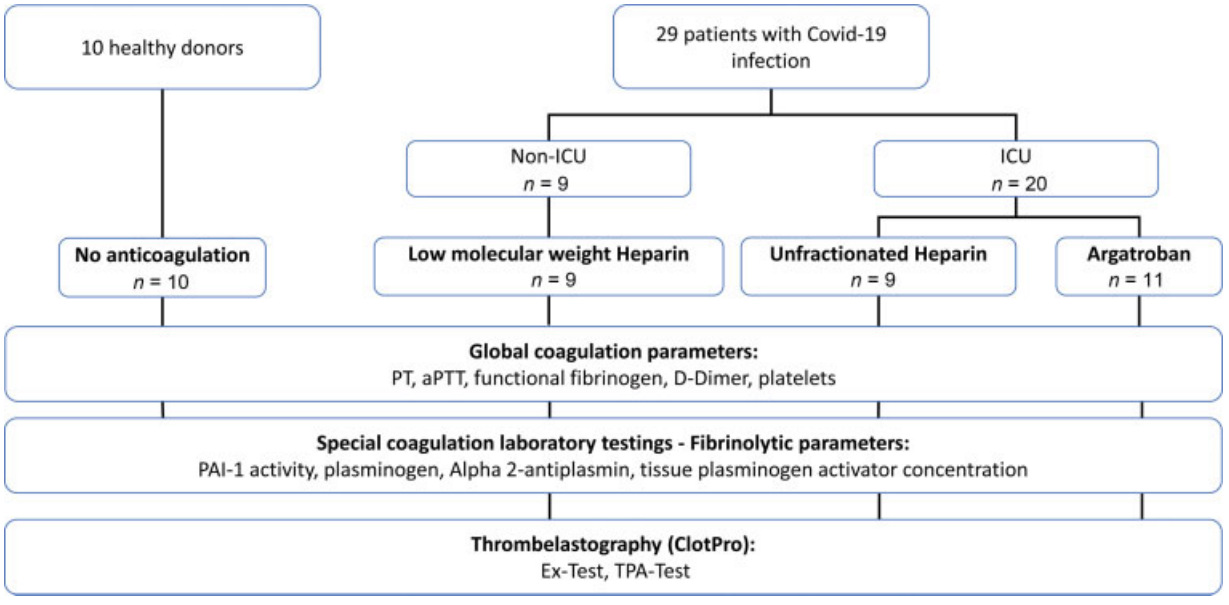

Fig. 1 Flow chart of included COVID-19 patients and healthy donors. Blood samples were collected from 29 consecutive patients with polymerase chain reaction (PCR) confirmed COVID-19 infection who were admitted to our hospital (aPTT, activated partial thromboplastin time; ICU, intensive care unit; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time).

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Table 1 Demographic characteristics and laboratory parameters of coagulation and fibrinolysis of patients included in the study (samples were obtained at the same time point for coagulation and fibrinolysis parameters)

Demographic characteristics								
			Healthy donors ($n = 11$)		**COVID-19**			
					Non-ICU ($n = 9$)		**ICU ($n = 20$)**	
Gender (n, %)			f = 5	(45%)	f = 5	(56%)	f = 2	(10%)
Age (y)			38	(25–47)	44	(14–76)	60 (55–75)	
Risk factors for severe COVID-19 disease		Diabetes mellitus	n.d.		1	(11%)	3	(15%)
		Hypertension	n.d.		4	(44%)	10	(50%)
		Obesity	n.d.		2	(22%)	6	(30%)
		Body mass index	n.d.		27.5	(24–33.7)	27.5	(25–30.4)
		Coronary artery disease	n.d.		2	(22%)	2	(10%)
Medication		Aspirin	n.d.		2	(22%)	2	(10%)
		Statin	n.d.		2	(22%)	5	(25%)
Laboratory parameters of coagulation and fibrinolysis								
	Healthy donors ($n = 11$)	**COVID-19**						
		Non-ICU		**ICU**		**ICU**		
		$n = 9$	***p*-Value[a]**	**$n = 20$**	***p*-Value[b]**	**UFH ($n = 9$)**	**Argatroban ($n = 11$)**	***p*-Value[c]**
PT (%)	n.d.	86.0, $n = 8$, (76.5–100.8)	n.d.	53.5 (41.5–71.3)	n.d.	69.0 (55.0–80.5)	43.0 (38.0–53.0)	0.0020
INR	n.d.	1.1, $n = 8$, (1.0–1.1)	n.d.	1.4 (1.2–1.6)	n.d.	1.2 (1.1–1.3)	1.6 (1.4–1.7)	0.0240
aPTT (s)	n.d.	23.0 (22.3–25.8)	n.d.	50.5 (41.8–59.8)	n.d.	57.0 (40.0–90.0)	47.0 (44.0–57.0)	0.2596
Functional fibrinogen (mg/dL)	294 (252.0–384.0)	424.0 (304.5–699.5)	0.0375	428.5 (257.3–504.8)	0.0152	504.0 (339.5–587.0)	334.0 (253.0–483.0)	0.1002
D-dimer (μg/mL)	n.d.	2.9 (1.1–3.6)	n.d.	3.1 (2.0–6.5)	n.d.	2.8 (1.8–6.4)	3.2 (1.8–7.1)	0.5273
Platelets (10^3/μL)	n.d.	253 (164.5–460)	n.d.	196 (104.5–273.0)	n.d.	202 (85.5–230)	190 (130–336)	0.4207
Fibrinolytic parameters								
PAI-1 activity (U/mL)	1.4 (0.4–2.2)	1.8 (0.2–4.2)	0.2332	3.8 (1.9–7.0)	0.0010	4.1 (2.2–10.3)	3.7 (1.7–6.4)	0.3711
Plasminogen (%)	107.0 (102.0–115.0)	116 (99.5–128.5)	0.4234	111 (85–134.8)	0.7796	97 (80–121)	120 (100–149)	0.4537
α2-antiplasmin (%)	106 (101.0–115.0)	106.0 (99.5–129.5)	0.6855	94.5 (76.3–102.5)	0.0024	87.0 (76.0–99.0)	95.0 (79.0–113.0)	0.1578
tPA (ng/mL)	1.2 (0.8–1.8), $n = 10$	2.4 (1.5–5.4), $n = 8$	0.0441	3.2 (1.5–6.2), $n = 19$	0.0006	5.3 (2.0–7.7)	2.3 (1.2–5.3) [$n = 10$]	0.1956

Abbreviations: aPTT, activated partial thromboplastin time; f, female; ICU, intensive care unit; INR, international normalized ratio; n.d., not determined; n, number of patients included; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; tPA, tissue plasminogen activator; UFH, unfractionated heparin.
Note: Data are presented in total number (percentage) or in median (interquartile range).
[a]Non-ICU patients are compared with healthy donors.
[b]ICU patients are compared with healthy donors.
[c]ICU patients anticoagulated with heparin are compared with ICU patients anticoagulated with argatroban.

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of 20 (15%) ICU COVID-19 patients had a spontaneous respiration and 8/20 (40%) patients needed additionally venous-venous extracorporeal membrane oxygenation (vv-ECMO). Fourteen of 20 (70%) patients had known risk factors for severe manifestation of COVID-19 infection as described previously,[2] including hypertension in 10/20 (50%) patients, obesity in 6/20 (30%) patients, coronary artery disease in 2/20 (10%) patients and diabetes mellitus in 3/20 (15%) patients. Five of 20 (25%) patients on ICU died within the follow-up period of 30 days. Anticoagulation on ICU patients was performed using unfractionated heparin (UFH) in 9/20 (45%) and argatroban in 11/20 (55%) patients.

Routine Laboratory Investigations of the Coagulation System

The PLT of ICU COVID-19 patients showed no significant reduction with 196×10^9/L platelets (IQR: 105–273×10^9/L) compared with non-ICU patients with 253×10^9/L platelets (IQR: 165–460×10^9/L, $p = 0.12$). The level of D-dimer and the level of functional fibrinogen of ICU COVID-19 patients did not show significant differences with a median concentration of D-dimer of 3.05 µg/mL (IQR: 1.95–6.5 µg/mL) and functional fibrinogen of 428 mg/dL (IQR: 257–504 mg/dL) compared with non-ICU patients with 2.9 µg/mL (IQR: 1.1–3.6 µg/mL, $p = 0.1377$) and 424 mg/dL (IQR: 305–700 mg/dL, $p = 0.3173$), respectively. The PT was significantly decreased in COVID-19 patients in ICU compared with non-ICU patients (54% [IQR: 42–71%] vs. 86% [IQR 77–101%], $p = 0.015$). Also, the aPTT was significantly longer in ICU than in non-ICU patients (50.5 seconds [IQR: 41.75–59.75 seconds] vs. 23 seconds [IQR: 22.25–-25.75 seconds], $p < 0.0001$; ►**Table 1**).

Effects of Anticoagulation on Standard Coagulation and Viscoelastic Parameters

Heparin and argatroban are the standard medications that are used in our hospital for anticoagulation in COVID-19 infections. In routine diagnostics, the UFH dosage is adjusted according to aPTT. Argatroban is also administered by an aPTT controlled regimen. Changes in thromboelastographic parameters are reported to correlate with the level of argatroban. So in critically ill patients, TEG parameters may provide better correlation to argatroban plasma concentrations than aPTT.[18] The concentration of unfractionated heparin is best correlated with the level of anti-Xa and the R time of TEG.[19] The R time in TEG corresponds to the CT of the EX test in ClotPro.

In our study, heparin treatment prolonged the CT. On the other hand, argatroban resulted in a significantly longer CT and CFT than anticoagulation with heparin (►**Table 2**). The α angle, describing the capacity of thrombin generation, was significantly reduced in patients anticoagulated with argatroban compared with those treated with heparin (►**Table 2**).

COVID-19-related Main Findings

Severe COVID-19 Infection is Associated with Reduced Ability of Clot Resolution

The spontaneous clot lysis in the Ex test was significantly reduced in ICU COVID-19 patients (Ex test, maximal clot lysis, ICU COVID-19 patients vs. healthy donors: 3.25 ± 0.56 vs. $6.2 \pm 0.89\%$, $p = 0.013$; ►**Fig. 2A**). No significant reduction was observed in non-ICU COVID-19 patients compared with healthy controls (Ex test, maximal clot lysis, non-ICU COVID-19 patients vs. healthy donors: 5.56 ± 1.23 vs. $6.2 \pm 0.89\%$, $p = 0.68$; ►**Fig. 2A**). A representative example for reduced clot lysis is shown in ►**Figs. 2B** and **C**.

Blood samples from ICU COVID-19 patients as well as from non-ICU COVID-19 patients showed significantly longer time to clot lysis after the addition of exogenous tPA compared with healthy donors (TPA test, LT, ICU COVID-19 patients vs. healthy donors: 365.7 ± 44.6 vs. 193.2 ± 16.3 seconds, $p = 0.0014$; non-ICU COVID-19 patients: 354.3 ± 30.5 seconds, $p = 0.0005$; ►**Fig. 2D**). A representative example for prolonged time to clot lysis is shown in ►**Fig. 2E, F**.

No significant differences were observed when results of CFT, α angle and MCF were compared between ICU COVID-19 patients and healthy donors. CT was significantly prolonged in ICU COVID-19 patients due to therapeutic anticoagulation. Data are summarized in ►**Table 2**. Taken together, these data indicate that severe COVID-19 is associated with a hypofibrinolytic state in blood.

Severe COVID-19 Infection Induces Impairment of the Fibrinolysis System

To investigate the mechanism of the reduced fibrinolysis activity, we determined the plasma levels of tPA and the activity of plasminogen, PAI-1 and A2AP. The concentration of the profibrinolytic factor tPA was significantly higher in plasma samples from non-ICU and ICU COVID-19 patients compared with healthy donors (non-ICU COVID-19 patients vs. healthy donors: 3.13 ± 0.69 vs. 1.39 ± 0.23 ng/mL, $p = 0.0441$; ICU COVID-19 patients: 3.97 ± 0.61 ng/mL, $p = 0.0006$, respectively; ►**Fig. 3A**). The A2AP activity was significantly decreased in ICU COVID-19 patients compared with healthy donors (91.6 ± 4.1 vs. $107.3 \pm 2.2\%$, $p = 0.002$; ►**Fig. 3B**). However, similar plasminogen activity was measured in plasma samples (non-ICU COVID-19 patients vs. healthy donors: 125.0 ± 13.7 vs. $112.4 \pm 6.5\%$, $p = 0.42$; ICU COVID-19 patients: $115.5 \pm 8.8\%$, $p = 0.78$; ►**Fig. 3C**). Interestingly, ICU COVID-19 patients had significantly higher concentrations of PAI-1 activity compared with healthy donors ((4.92 ± 0.91 vs. 1.28 ± 0.33 U/mL, $p = 0.001$; ►**Fig. 3D**). In addition, the time to resolve 50% of the formed clot after addition of exogenous tPA was positively correlated with the PAI-1 activity ($r = 0.70$, $p = 0.0006$; ►**Fig. 3E**). In fact, patients with a pathologically longer LT in TPA test, defined as more than twice the standard deviation added to the median LT of healthy donors, showed significantly higher levels of PAI-1 activity compared with patients with values within the normal range (6.56 ± 1.31 vs. 2.91 ± 0.92 U/mL, respectively, $p = 0.036$; ►**Fig. 3F**). Data are summarized in ►**Table 1**.

The Association between the Thromboelastographic Properties and Clinical Manifestations in Severe COVID-19 Infections

Six (6/20, 30%) patients with severe COVID-19 had thrombotic events within 30 days and 5/20 (25%) patients deceased

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Table 2 Summary of the viscoelastic parameters of the formed clot in blood of COVID-19 patients

	Healthy donors ($n = 10$)	COVID-19						
		Non-ICU		ICU		ICU		
		$n = 9$	*p*-Value[a]	$n = 20$	*p*-Value[b]	UFH ($n = 9$)	Argatroban ($n = 11$)	*p*-Value[c]
VT: ex test								
Clotting time (s)	57.5 (51.3–61.5)	51.0 (45.0–55.5)	0.6257	127.0 (70.3–215.0)	0.0001	71.0 (61.5–97.0)	203.0 (140.0–222.0)	0.0007
Clot formation time (s)	55.0 (54.3–57.8)	30.0 (26.5–41.0)	< 0.0001	61.0 (52.0–101.3)	0.0891	52.0 (43.5–61.0)	90.0 (57.0–120.0)	0.0545
α angle (degrees)	77.0 (76.0–77.0)	81.0 (79.5–83.0)	< 0.0001	78.0 (70.3–79.0)	0.1822	78.0 (76.3–79.5)	71.0 (68.0–78.0)	0.0500
Maximum of clot firmness (mm)	61.0 (59.5–63.0)	64.0 (63.0–71.5)	0.0260	67.0 (55.3–69.0)	0.2341	65.0 (58.5–68.0)	68.0 (54.0–70.0)	0.8541
Maximum lysis (%)	6.5 (3.0–8.0)	7.0 (2.0–8.0)	0.6773	4.0 (1.0–5.0)	0.0127	4.0 (0.5–6.0)	3.0 (1.0–5.0)	0.6373
VT: TPA test								
Clotting time (s)	41.0 (39.5–50.8)	38.0 (33.0–43.0)	0.8869	108.5 (53.0–192.0)	0.0003	53.0 (44.5–81.5)	180.0 (115.0–224.0)	0.0025
Clot formation time (s)	66.0 (60.8–80.5)	47.0 (36.5–56.0)	0.0002	65.0 (52.0–78.0), $n = 15$	0.8420	62.0 (49.5–72.5), $n = 9$	69.0 (57.8–92.3), $n = 6$	0.2763
α angle (degrees)	76.5 (73.8–78.3)	80.0 (79.5–83.0)	0.0002	77.5 (62.5–80.0)	0.0840	79.0 (76.0–80.0)	67.0 (45.0–79.0)	0.0191
Maximum of clot firmness (mm)	27.0 (24.0–30.0)	45.0 (39.5–61.0)	0.0005	41.5 (17.3–52.3)	0.0790	46.0 (37.0–51.5)	24.0 (10.0–54.0)	0.0361
Maximum lysis (%)	94.5 (94.0–95.0)	97.0 (96.0–97.5)	< 0.0001	96.0 (86.0–97.0)	0.1459	96.0 (91.0–97.0)	92.0 (83.3–97.0)	0.3970
Time of lysis (s)	177.5 (169.3–195.0)	310.0 (291.5–435.5)	0.0005	299.0 (237.8–475)	0.0014	335.0 (280.5–625.0)	288.0 (190.0–333.0)	0.1229

Abbreviations: Ex test, extrinsic assay; ICU, intensive care unit; *n*, number of patients included; TPA-test, tissue plasminogen activator added to extrinsic assay; UFH, unfractionated heparin; VT, viscoelastic test assay.
Note: Data are presented in median (interquartile range).
[a]Non-ICU patients are compared with healthy donors.
[b]ICU patients are compared with healthy donors.
[c]ICU patients anticoagulated with heparin are compared with ICU patients anticoagulated with argatroban.

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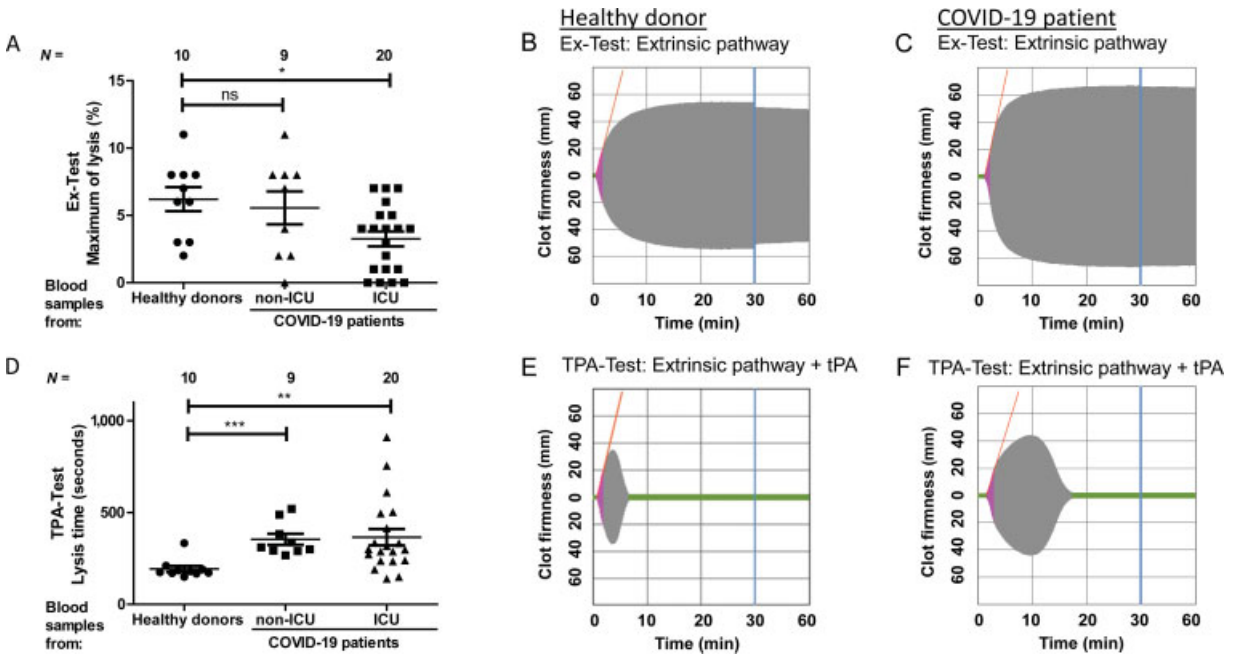

Fig. 2 Viscoelastic properties of clot formed in blood samples from COVID-19 patients. Citrated blood samples were collected and investigated within 2 hours using a viscoelastic test system (ClotPro Enicor GmbH, Munich, Germany). (**A**) The maximum lysis in the ex test. Representative thromboelastographs showing an increased stability of the formed clots from blood samples of (**B**) COVID-19 patients and (**C**) healthy donors. (**D**) The lysis time in the TPA test. Representative thromboelastographs showing a delayed resolution of the clots from blood samples of (**E**) COVID-19 patients and (**F**) healthy donors. Data are presented as mean ± SEM of the measured fold increase compared with control (ns, not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$).

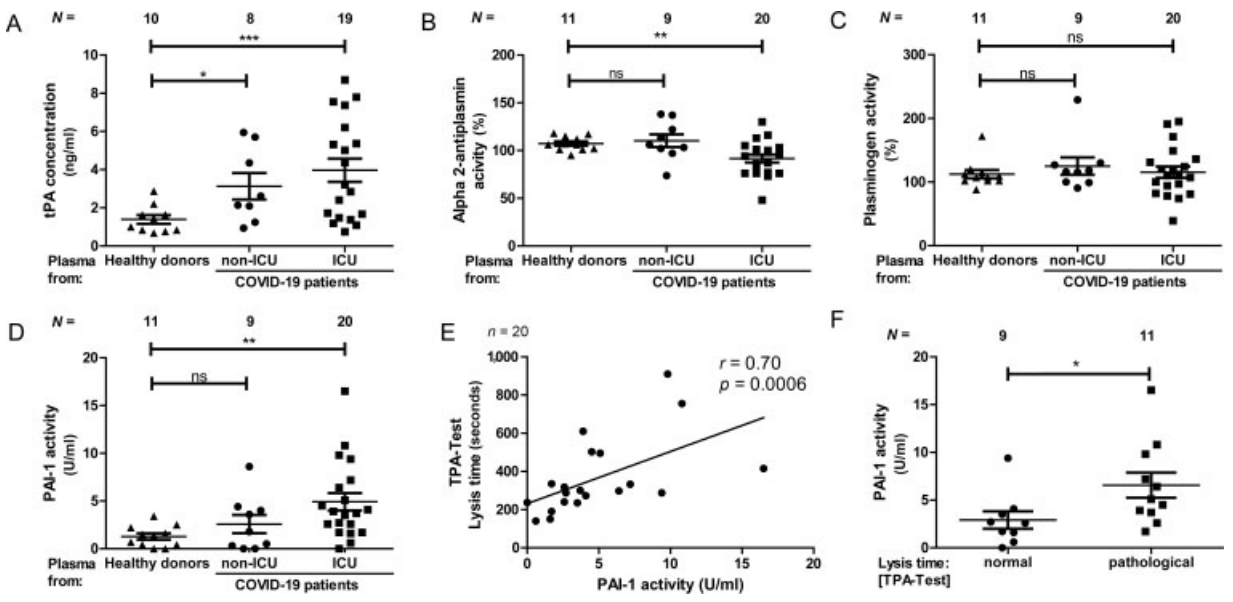

Fig. 3 Plasma concentrations and interactions of factors of the fibrinolysis system. The plasma concentration of (**A**) tissue plasminogen activator (tPA), (**B**) α2-antiplasmin (A2AP), (**C**) plasminogen activity and (**D**) plasminogen activator inhibitor-1a (PAI-1) were measured using commercially available assays. (**E**) The correlations between PAI-1 and the lysis time (LT) in the TPA test of viscoelastic investigation measured on the same day. (**F**) A significant increased level of PAI-1 activity was observed in intensive care unit (ICU) patients with a pathologically longer LT (defined as the double standard deviation added to the median LT in healthy donors) than in ICU patients with an LT in references. Data are presented as mean ± SEM of the measured fold increase compared with control (ns, not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$).

within 10 weeks of treatment in ICU. No significant differences were observed in viscoelastic results regarding thrombosis or mortality. Additionally no association between age, gender or body mass index (BMI) and the thromboelastographic parameters was found (data are not shown).

Discussion

Our study show that severe COVID-19 is associated with a hypofibrinolytic state in blood, where fibrinolytic inhibitors are elevated leading to increased resistance to clot lysis. Our

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findings suggest that the fibrinolysis system significantly contributes to the procoagulatory status in COVID-19 patients and might represent a therapeutic target.

Accumulating evidence suggests that a hypercoagulable state exists in patients with COVID-19, leading to increased risk of thromboembolic complications. Under physiological conditions, the fibrinolysis system counters hyperactivation of the coagulation system to prevent uncontrolled propagation of fibrin disposition in vessels.

In our study, we observed a reduced activity of the fibrinolytic system. Blood samples from severe COVID-19 patients showed a significant reduction of the spontaneous clot lysis after activation of the extrinsic coagulation pathway. Most importantly, an increased resistance to tPA was observed in all COVID-19 patients (non-ICU and ICU patients) as reflected by the longer time to clot resolution in the TPA test. This finding indicates that clots formed during SARS-CoV-2 infection may have different structures that prevent sufficient fibrinolysis. Another explanation could be the reduced fibrinolysis activity in plasma from COVID-19 patients. The fibrinolytic inhibitor in plasma, PAI-1, which is known to be elevated in severe acute respiratory syndrome,[20] was also increased in our cohort of COVID-19 patients. Plasma PAI-1 levels have also been reported as an independent risk factor for poor prognosis and mortality in acute respiratory distress syndrome (ARDS). PAI-1 is released from endothelial cells and platelets, the major circulating pool of PAI-1, upon activation.[21,22] Recent reports demonstrate viral inclusions within endothelial cells and sequestered mononuclear and polymorphonuclear cellular infiltration, with evidence of endothelial apoptosis in the post mortem of SARS-CoV-2 infection.[23] We and others showed that severe COVID-19 infection is associated with increased platelet activation.[24,25] Our data show that tPA levels are indeed increased in COVID-19 infections. However, the expected increase of plasminogen activity was not observed. Most importantly, there was no hyperfibrinolysis, but decreased clot lysis and resistance to tPA were found. Together with the elevated levels of PAI-1, these data indicate that PAI-1 overcomes the functionality of the fibrinolytic system despite the increased release of tPA.

Our observation might have several clinical implications. Patients with severe COVID-19 infection are immobilized, which is an additional risk factor for hospital-associated VTE.[26,27] Therefore, a prophylactic anticoagulant therapy with LMWH is recommended for hospitalized patients with COVID-19, without contraindications for heparin therapy. Recently, we reported on thromboembolic complications in two COVID-19 patients who received therapeutic anticoagulation.[28] Although anticoagulant therapy might be helpful to limit the extent of the coagulopathy, this therapeutic approach alone might be ineffective in clearing fibrin clusters deposited due to the increased levels of PAI-1 in COVID-19.

Our data underline the general observation; the pathophysiology of COVID-19-associated thromboembolic complications seems to be complex and multifactorial. Of note, our patients developed thrombosis despite therapeutic anticoagulation during the ICU stay, indicating a requirement to readdress the balance of fibrinolysis in severe COVID-19 patients, by either enhancing plasminogen activation or downregulating fibrinolytic inhibitors. In fact, a recent study investigated longitudinal samples from COVID-19 patients and showed an almost elevated PAI-1 activity in COVID-19 patients.[29] Also, an increased level of PAI-2, but not of tPA, followed by an inhibition of fibrinolysis in patients who did not survive COVID-19 infection has been described.[30] It is worth noting that a clinical trial revealed a significant improvement in patients with severe ARDS secondary to trauma or sepsis after administration of urokinase plasminogen activator (uPA) or streptokinase.[31] In addition, recent studies showed that fibrinolytic therapy can improve survival in ARDS[27] and might be beneficial for COVID-19 patients.[26] Although the optimal dosing regimen is still to be determined,[26] these data may suggest that tPA might be a promising approach awaiting effective treatments for COVID-19.

From a diagnostic point of view, the advantage of the viscoelastic test systems is their wide use as point-of-care methods in many fields of intensive care to guide haemostatic treatment in patients with bleeding.[3] Our data suggest that these assays could be used to monitor the efficacy of anticoagulation as wells as fibrinolytic treatment and to predict thromboembolic complications in COVID-19 patients. Recent studies have already described hypercoagulability and resistance to fibrinolysis,[13] but no comparison to fibrinolytic parameters was ever shown. The determination of the PAI-1 and tPA level is important because higher levels of PAI-1 and tPA are associated with worse outcome in COVID-19 patients.[10,11] However, more studies are needed to improve the diagnostic performance of the viscoelastic tests, since the extent of ML does not completely allow a fine and reliable quantification of the fibrinolysis and clot retraction could also contribute to the secondary shortening of the amplitude.

Our study is subjected to some limitations. First, as an observational, monocentric study, we cannot conclude that the reported associations between anti-fibrinolytic markers in COVID-19 are causal or specific to the disease. Second, we cannot exclude the possibility of remaining residual confounding or unmeasured potential confounders, as for the increase of PAI-1 in ARDS. Third, the small sample size does not enable a final and robust statistical analysis to assess clinical outcomes. Larger cohorts should be investigated in the future to confirm this observation. Finally, it should be noted that it is difficult to identify specific changes in coagulation and fibrinolysis system in severely ill patients by the PT, INR, aPTT, fibrinogen, D-dimer and PLT or viscoelastic tests. Nevertheless, data presented in this study may provide a background for future studies to dissect mechanisms related to the fibrinolysis system that might be involved in the progression of COVID-19.

Conclusion

In our study, we observed reduced activity of the fibrinolytic system. Blood samples from severe COVID-19 patients showed a significant reduction of the spontaneous clot lysis after activation of the extrinsic coagulation pathway and an increased resistance to tPA as reflected by the longer time to

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clot resolution. These findings may suggest a potential link between fibrinolysis impairment and thrombosis. These data may indicate fibrinolysis as a potential target of COVID-19 treatment and emphasize the need for large-scale multicentre study to investigate the predictive power of clot viscoelastic properties in well-phenotyped longitudinal cohorts.

What is known about this topic?

- COVID-19 infection is associated with procoagulatory state.
- Thromboelastographic investigations are useful point-of-care devices to assess coagulation and fibrinolysis.

What does this paper add?

- Blood samples from severe COVID-19 patients showed a significant reduction of the clot lysis.
- PAI-1 activity was significantly higher in plasma samples of ICU COVID-19 patients and positively correlated with the clot LT.

Author Contributions

S.H., Ch.Sch., H.H. and T.B. designed the study. S.H., K.A. and A.P. performed the experiments. S.H., K.A., P.M. and T. B. collected and analyzed the laboratory data. S.H., H.H., M.B., R.H., N.P.M., P.L., V.M., T.G., M.G. and T.B. collected and analyzed the clinical data. All authors approved the final version of the manuscript.

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Conflict of Interest

None declared.

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