ANESTHESIOLOGY

Greater Fibrinolysis Resistance but No Greater Platelet Aggregation in Critically Ill COVID-19 Patients

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Although critically ill patients with COVID-19 are at an increased risk for thromboembolic complications, the details of hemostatic balance regarding clot lysis and platelet contribution to clot formation are not well understood.

What This Article Tells Us That Is New

- Despite increases in von Willebrand factor, platelet aggregability based on impedance aggregometry testing was not increased in critically ill COVID-19, although viscoelastometric testing noted fibrinolysis resistance. These findings contribute to our understanding of the hypercoagulable state of COVID-19 and may have important considerations for management strategies.

ABSTRACT

Background: The hemostatic balance in patients with coronavirus disease 2019 (COVID-19) seems to be shifted toward a hypercoagulable state. The aim of the current study was to assess the associated coagulation alterations by point-of-care-diagnostics, focusing on details of clot formation and lysis in these severely affected patients.

Methods: The authors' prospective monocentric observational study included critically ill patients diagnosed with COVID-19. Demographics and biochemical data were recorded. To assess the comprehensive hemostatic profile of this patient population, aggregometric (Multiplate) and viscoelastometric (CloPro) measures were performed in the intensive care unit of a university hospital at a single occasion. Coagulation analysis and assessment of coagulation factors were performed. Data were compared to healthy controls.

Results: In total, 27 patients (21 male; mean age, 60 yr) were included. Impedance aggregometry displayed no greater platelet aggregability in COVID-19 in comparison with healthy controls (area under the curve [AUC] in adenosine diphosphate test, 68 ± 37 U *vs.* 91 ± 29 U [–27 (Hodges–Lehmann 95% CI, –48 to –1); $P = 0.043$]; AUC in arachidonic acid test, 102 ± 54 U *vs.* 115 ± 26 U [–21 (Hodges–Lehmann 95% CI, –51 to 21); $P = 0.374$]; AUC in thrombin receptor activating peptide 6 test, 114 ± 61 U *vs.* 144 ± 31 U [–31 (Hodges–Lehmann 95% CI, –69 to –7); $P = 0.113$]). Comparing the thromboelastometric results of COVID-19 patients to healthy controls, the authors observed significant differences in maximum clot firmness in fibrin contribution to maximum clot firmness assay (37 ± 11 mm *vs.* 15 ± 4 mm [21 (Hodges–Lehmann 95% CI, 17 to 26); $P < 0.001$]) and lysis time in extrinsic activation and activation of fibrinolysis by tissue plasminogen activator assay (530 ± 327 s *vs.* 211 ± 80 s [238 (Hodges–Lehmann 95% CI, 160 to 326); $P < 0.001$]).

Conclusions: Thromboelastometry in COVID-19 patients revealed greater fibrinolysis resistance. The authors did not find a greater platelet aggregability based on impedance aggregometric tests. These findings may contribute to our understanding of the hypercoagulable state of critically ill patients with COVID-19.

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Coronavirus disease 2019 (COVID-19) still poses a critical threat to global health. The number of patients infected with SARS-CoV-2 surpassed almost three million in April 2020, and the global death rate is constantly increasing. The clinical manifestations range from asymptomatic or very mild to severe disease and death.

Several case series and cohort studies have described abnormal coagulation parameters in COVID-19–infected patients and have shown that excessive coagulation activation has prognostic relevance with regard to hospital mortality and the need for intensive care.[1–3] These findings are supported by published data describing a high incidence of venous thromboembolism in up to 31% of critically ill cases.[4] Current recommendations therefore suggest considering early anticoagulation to prevent thromboembolism.[5,6] The underlying causes for the reported enhanced risk of thromboembolic events and hypercoagulability are not yet known. We therefore conducted this study to better characterize the

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COVID-19–related coagulation changes using aggregometric (Multiplate; Roche Diagnostics, Switzerland) and viscoelastic (ClotPro; enicor GmbH, Germany) testing as well as a comprehensive determination of coagulation factors.

Based on reports from China, Italy, and the United States, patients diagnosed with COVID-19 suffer from hypercoagulability during the course of their disease. We hypothesize that coagulation alterations may be assessed by point-of-care diagnostic tools, and we sought to provide further information on the underlying pathology by focusing on the details of clot formation and lysis as a decisive element in the pathogenesis of thromboembolism. This study aimed to provide insights into the characteristics of hypercoagulability in these patients.

Materials and Methods

Patients diagnosed with COVID-19 and admitted to the intensive care unit (ICU) of the authors' institution were included into this prospective, monocentric observational study. The inclusion criteria were age greater than or equal to 18 yr, moderate to severe acute respiratory distress syndrome (ARDS) due to SARS-CoV-2 infection, and no history of congenital, acquired, or any other known coagulopathy.

The study was performed in accordance with the Declaration of Helsinki. Approval from the local ethics committee was obtained before the study was conducted (No. 20-643), and a waiver regarding the requirement of written informed consent from COVID-19 patients was authorized. All participants of the control group provided written informed consent and were recruited only for the current study. Patient care and study conduct complied with good clinical practice.

Demographic and biochemical data as well as the medical history of patients admitted for COVID-19 were recorded. A healthy control population comprising volunteers without previous history of hyper- or hypocoagulable disorders was examined for this study. These individuals were recruited concurrently with the patients from the community between April 1 and April 15, 2020.

Thromboembolic Prophylaxis and Intensive Care of COVID-19 Patients

Upon admission to the ICU, all patients received mechanical ventilation and critical care therapy as put forth by Poston *et al.*[7] The regimen of thrombosis prophylaxis was 60 mg (or 80 mg at body mass index greater than 35 kg/m^2) of low-molecular-weight heparin (calcium enoxaparin) twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin with a target activated partial thromboplastin time (PTT) of 50 to 70 s. Antithrombin (AT) concentrate was replaced regularly to maintain a level of 80% or greater. No additional drugs with known antiplatelet effects were taken other than indicated. No experimentally intended antiviral therapies (remdesivir, hydroxychloroquine, or other antiviral agents) were applied.

Laboratory Analyses

Venous blood was collected *via* a cannula inserted into a cubital vein. Collection tubes for conventional coagulation analysis were prefilled with sodium citrate (S-Monovette 1.8 ml, sodium-citrate 3.2% [1:10]; Sarstedt AG, Germany) and analyzed by an ACL Top 700 CTS (Werfen GmbH, Spain). Hematological analyses were performed using collection tubes prefilled with ethylenediaminetetraacetate (S-Monovette 1.6 ml, K3 EDTA; Sarstedt AG) and analyzed by an XN 9000 (Sysmex GmbH, Germany). Platelet count was determined by fluorescence flow cytometry on an XE 2100 (Sysmex GmbH), and biochemical parameters were assessed using serum collection tubes (S-Monovette 7.5 ml, Serum Gel with clotting activator; Sarstedt AG) and analyzed by a Cobas 8000 (Roche Diagnostics, Germany).

For ClotPro analysis, blood was collected into collection tubes prefilled with sodium citrate (S-Monovette 1.8 ml, citrate 3.2% [1:10]; Sarstedt AG). For multiple electrode aggregometry analysis, a heparinized blood gas analysis sample tube (safePICO; Radiometer, Germany) was used.

Multiple Electrode Aggregometry

Platelet function was measured by multiple electrode aggregometry using the Multiplate analyzer 15 min after blood draw and after activation with commercially available standard reagents (Roche, Basel, Switzerland) as previously published.[8] Blood samples were analyzed at 37°C. To test different methods of aggregation induction, aggregation was stimulated *via* (1) adenosine diphosphate ([ADP] 6.4 mmol/l) receptors by ADP; (2) arachidonic acid, the substrate of cyclooxygenase (0.5 mmol/l arachidonic acid), which subsequently forms the potent platelet activator thromboxane A_2; and (3) thrombin receptor activating peptide 6 (32 mmol/l) *via* the platelet surface platelet receptor as described previously.[8] To identify abnormal values in multiple electrode aggregometry assays, reference ranges were defined in accordance with the manufacturer's recommendations for heparinized blood samples.[9]

Thromboelastometry

Thromboelastometric assays were performed 15 min after blood draw. Blood samples were analyzed at 37°C using ClotPro analyzer (enicor GmbH, Haemonetics, Germany).

The ClotPro analyzer provides bedside viscoelastometric measurements of whole blood coagulation by recording kinetic changes in a sample of citrated whole blood, similar to rotational thromboelastometry (ROTEM).[10,11] The blood sample is placed into a cylindrical cup, which rotates alternately. A stationary cylindrical pin is then inserted into the cup. The clotting sample reduces the movement of the cup gradually as the clot firmness rises. The cup movement

is recorded and transformed into an amplitude, which is continuously recorded against the time and expressed in millimeters for historical reasons.

The run time is set to 40 min and automatically stopped by the software. Regular quality control tests were run in accordance with the manufacturer's instructions. For the current study, four tests were carried out using reagents provided by the manufacturer: recombinant tissue factor–triggered extrinsic pathway, which evaluates the extrinsic pathway; ellagic acid–activated intrinsic pathway, which evaluates the intrinsic pathway; cytochalasin D and synthetic glycoprotein IIb/IIIa antagonist, which are inhibitors of the rearrangement of microtubules in platelets and thus of platelet aggregation, which evaluates the contribution of fibrin to clot firmness; and tissue factor–triggered extrinsic pathway and activation of fibrinolysis by high-dose (650 ng/ml) recombinant tissue plasminogen activator (Tpa), which reflects resistance to fibrinolysis. All tissue factor–triggered assays contain polybrene as an antagonist of heparin present in the sample.

When performing viscoelastic tests, the following parameters were calculated: clotting time, which is the time from the start of the test until the clotting of the sample (2-mm clot firmness), expressed in seconds; clot formation time, which is the time from clotting time until an amplitude of 20 mm is detected, expressed in seconds; maximum clot firmness expressed in millimeters; maximum lysis of the clot in percentage of the maximum clot firmness; and the lysis time, which is the time from the end of clotting time until a lysis of 50% of the maximum clot firmness is recorded, expressed in seconds. The maximum lysis reflects the percentage of lysis in relation to the maximum clot firmness. For technical reasons, the lowest amplitude during lysis is 1.5 mm (*i.e.*, if an amplitude of less than 1.5 mm is reached, the amplitude is still displayed as 1.5 mm). In conclusion, maximum lysis cannot reach 100%. To identify abnormal values in thromboelastometric assays, reference ranges were defined in accordance with the manufacturer's instructions. The reference range for lysis time as given by the manufacturer ranges from 145 to 438 s.

Statistical Analysis

No statistical power calculation was conducted before the study. The sample size was based on the available data. The current study is a *post hoc* analysis. Data were tested for normality using the Kolmogorov–Smirnov test. Data comparisons of patient characteristics were made using the Student's *t* test; results of both multiple electrode aggregometry and thromboelastometry were made using the Mann–Whitney U test and Hodges–Lehmann estimator. Adjusted analysis was performed for the three main potential confounders. Here a stratified nonparametric approach was used for sex and a linear regression for the continuous confounders age and body mass index.

All statistical tests were two-tailed, and results with $P < 0.05$ were considered statistically significant. All calculations/analyses were performed with SPSS (Version 25; IBM, USA), R software (Version 3.6; The R Foundation, USA), and GraphPad Prism (Version 8; GraphPad, USA). There were no missing data.

Results

Patient Characteristics

The patient population included 27 patients diagnosed with COVID-19 and ARDS who were treated in our ICU. The demographic data are presented in table 1. Overall, 21 of the patients were male. Of all patients, 25 (93%) were obese according to the definition of the World Health Organization, and six (22%) had a body mass index greater than 40 kg/m^2. The

Table 1. Patient Characteristics

	COVID-19 Patients (n = 27)	Healthy Controls (n = 12)	*P* Value
Age, yr (mean ± SD)	60 ± 13	38 ± 6	< 0.001
Male sex, n (%)	21 (78)	6 (50)	0.125
BMI, kg/m^2 (mean ± SD)	33.7 ± 7.6	24.5 ± 2.6	< 0.001
Comorbidities, n (%)			
Hypertension	14 (52)	0	0.006
Cardiovascular disease	7 (26)	0	0.155
Diabetes	11 (41)	0	0.018
Malignancy	3 (16)	0	0.548
Cerebrovascular disease	2 (24)	0	1
Chronic kidney disease	5 (18)	0	0.295
Mechanical ventilation, n (%)	27 (100)	0	< 0.001
Renal replacement therapy, n (%)	14 (52)	0	0.006
Simplified Acute Physiology Score II (mean ± SD)	42 ± 10	Not applicable	
Pa_{O_2}/Fi_{O_2} ratio at admission (mean ± SD)	138 ± 66	Not applicable	

Data are given as mean ± SD or count and percentage as indicated. Data comparisons of patient characteristics were made using Student's *t* test. BMI, body mass index; COVID-19, coronavirus disease 2019; Fi_{O_2}, fraction of inspired oxygen, Pa_{O_2}, partial pressure arterial oxygen.

mean age was 60 ± 13 yr. The mean ICU stay from admission until impedance aggregometric and viscoelastic assessment was 7 ± 3.5 days. The most frequent preexisting comorbidities were arterial hypertension (52%) and diabetes (41%). All patients were mechanically ventilated, and 14 patients (52%) received renal replacement therapy due to acute renal failure. To classify the severity of disease, the Simplified Acute Physiology Score II was assessed, revealing a mean score of 42.[10] The control group consisted of 12 healthy volunteers without any known preexisting conditions. The mean age of the control group was 38 ± 6 years; the mean body mass index was 24.5 ± 2.6 kg/m^2, and there were statistically significant differences in age ($P < 0.0001$), body mass index ($P < 0.0001$), prevalence of preexisting conditions, use of mechanical ventilation ($P < 0.0001$), and renal replacement therapy ($P < 0.0001$) between the COVID-19 patients and the control group.

Laboratory Parameters

The results of the coagulation parameters are presented in table 2. The median values revealed that PTT; AT and fibrinogen levels; platelet count; PTT; and activity of factor II, factor V, factor VII, factor XI, factor XII, and protein C were within the normal range in patients with COVID-19. In 12 patients (44%), AT was substituted as mentioned above. The mean cumulative dose was 2,500 IU (data not shown). Furthermore, we detected elevated D-dimer levels (3,656 ng/ml [interquartile range, 1,130 to 6,749]), elevated activity of factor VIII (261.8 ± 78.7%) factor IX (150.7 ± 53.3%), and von Willebrand factor (vWF) antigen (554 ± 109.7%), and lesser activity of factor XIII (64.5 ± 35.1%) and protein S (45 ± 30.1%). Thrombocytosis (defined as platelet count greater than 350 10^3/μl) was detected in 10 (37%) patients. Median D-dimer levels and activity of factor VIII, factor IX, and vWF antigen exceeded the upper reference limit. The median activity of factor X, factor XIII, and protein S was less than the lower reference limit.

Furthermore, we detected elevated median values of serum levels for c-reactive protein (16.7 ± 10.75 mg/dl), procalcitonin (1.7 ± 13.18 ng/ml), interleukin-6 (168 ± 904 pg/ml), ferritin (1,235 ± 423 ng/ml), and lactate dehydrogenase (494 ± 173 U/l), which are displayed in table 2.

Table 2. Laboratory Parameters

	COVID-19 Patients (n = 27)	Reference Range
C-reactive protein, mg/dl (IQR)	16.7 (10.75)	< 0.50
Lactate dehydrogenase, U/l (IQR)	494 (406–666)	< 248
Ferritin, ng/ml (IQR)	1,235 (4,231)	18–360
Procalcitonin, ng/ml (IQR)	1.7 (13.18)	< 0.50
Interleukin 6, pg/ml (IQR)	168 (904)	< 7
Quick, % (IQR)	83 (64–88)	70–130
International Normalized Ratio (IQR)	1.1 (1.0–1.35)	
Activated partial thromboplastin time, s (IQR)	34 (28–40)	25–37
Thrombin time, s (IQR)	16 (30.3)	10–17
Antithrombin, %	92 (72–109)	80–128
Fibrinogen, mg/dl (IQR)	467 (418–664)	190–498
Platelet count, 10^3/μl (IQR)	269 (178–365)	146–328
D-dimer, ng/ml (IQR)	3656 (1,130–6,749)	< 500
Factor II, % (IQR)	76.9 (60.4–88.7)	75–129
Factor V, % (IQR)	140.5 (121.4–163.4)	80–148
Factor VII, % (IQR)	71.4 (47.9–96.9)	48–139
Factor VIII, % (IQR)	261.8 (216.3–311.4)	68–133
Factor IX, % (IQR)	150.7 (107.3–185.6)	69–144
Factor X, % (IQR)	76.7 (56.6–99.7)	77–128
Factor XI, % (IQR)	127 (92–155)	76–155
Factor XII, % (IQR)	66.8 (43.4–90.7)	66–146
Factor XIII, % (IQR)	64.5 (52.1–101.9)	70–155
vWF antigen, % (IQR)	554 (431–600)	60–150
Protein C, % (IQR)	89 (77–109)	> 72
Protein S, % (IQR)	45 (34–62)	68–116

Data are given as medians. COVID-19, coronavirus disease 2019; IQR, interquartile range; vWF, von Willebrand factor.

Multiple Electrode Aggregometry

For impedance aggregometric assays, eight patients on therapy with acetylsalicylic acid were excluded for the arachidonic acid test, and one patient with thrombopenia was excluded from the analysis in accordance with Hanke *et al.*[11] demonstrating multiple electrode aggregometry results being dependent on platelet count.

In patients with COVID-19, impedance aggregometric assays were performed for AUC of ADP (68 ± 37 U), AUC of arachidonic acid (102 ± 54 U), and AUC of thrombin receptor activating peptide (114 ± 61 U). In 12 patients, the results of AUC for ADP were less than the lower reference range.

Comparing the multiple electrode aggregometry results of COVID-19 patients and healthy controls demonstrated significantly lower results for mean AUC for ADP (68 ± 37 U *vs.* 91 ± 29 U; −31 [95% CI, −48 to −1]; $P = 0.043$) in COVID-19 patients. No significant differences between COVID-19 patients and healthy controls were observed for mean AUC for arachidonic acid and mean AUC for thrombin receptor–activating peptide (table 3, fig. 1**)**. When checking for potential confounding effects of sex, age, and body mass index, the significance of the group difference for AUC for ADP may be explained by sex as it becomes insignificant after stratification.

Thromboelastometry

Thromboelastometric analyses are presented in table 3 and figure 2. In detail, thromboelastometry revealed values below the lower reference range for clot formation time in extrinsic activation in 2 of 27 patients, for clot formation time in intrinsic activation in 5 of 27 patients and for maximum clot firmness in intrinsic activation in 1 of 27 patients. Thromboelastometry displayed values greater than the reference range for clot formation time in extrinsic activation in no patient, for clot formation time in intrinsic activation in 4 of 27 patients, and for maximum clot firmness in intrinsic activation in 14 of 27 patients.

Table 3. Results of Impedance Aggregometric and Thromboelastometric Assays

	COVID-19 (n = 27)	Healthy Controls (n = 12)		
	Mean ± SD	Mean ± SD	Hodges–Lehmann Estimator of Shift (95% CI)	*P* Value
Impedance aggregometry*				
AUC adenosine-5 diphosphate, U	68 ± 37	91 ± 29	–27 (–48 to –1)	0.043
AUC arachidonic acid, U†	102 ± 54	115 ± 26	–21 (–51 to 21)	0.374
AUC thrombin receptor activator peptide 6, U	114 ± 61	144 ± 31	–31 (–69 to 7)	0.113
Thromboelastometry				
Extrinsic activation				
Clotting time, s	88 ± 22	60 ± 7	22 (15–33)	< 0.001
Clot formation time, s	59 ± 12	67 ± 18	–5 (–17 to 5)	0.265
Maximum clot firmness, mm	68 ± 5	57 ± 4	11 (8–14)	< 0.001
Intrinsic activation				
Clotting time, s	262 ± 120	163 ± 12	47 (25–92)	< 0.001
Clot formation time, s	100 ± 62	80 ± 13	–2 (–18 to 35)	0.915
Maximum clot firmness, mm	64 ± 8	56 ± 3	9 (5–13)	0.001
Contribution of fibrin to clot firmness				
Clotting time, s	104 ± 31	69 ± 14	28 (16–46)	< 0.001
Maximum clot firmness, mm	37 ± 11	15 ± 4	21 (17–26)	< 0.001
Extrinsic activation and activation of fibrinolysis by tPA				
Clotting time, s	68 ± 21	42 ± 9	25 (12–39)	< 0.001
Maximum clot firmness, mm	51 ± 12	26 ± 9	27 (19–33)	< 0.001
Maximum lysis, %	93 ± 15	92 ± 4	3 (2–5)	0.001
Lysis time, s	530 ± 327	211 ± 80	238 (160–326)	< 0.001

Data are presented as mean ± SD. Data comparisons were made using Mann–Whitney U Test and Hodges–Lehmann Estimator. *P* values are given for comparison between COVID-19 and healthy controls using Mann–Whitney U test.

*One patient was excluded due to thrombopenia. †Eight patients on therapy with acetylsalicylic acid were excluded from analysis.

AUC, area under the curve; COVID-19, coronavirus disease 2019; tPA, tissue plasminogen activator.

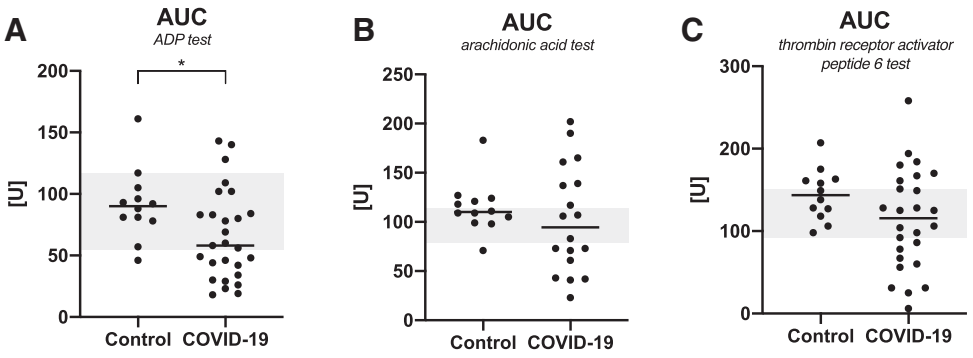

Fig. 1. Results of impedance aggregometry in COVID-19–infected patients and healthy controls. *Scatter plots* of impedance aggregometry. The *line* represents the median. The normal reference ranges of AUC are highlighted by a *gray area*. One patient was excluded due to thrombopenia. Eight patients on therapy with acetylsalicylic acid were excluded from analysis of AUC in arachidonic acid test. Data comparisons were made using Mann–Whitney U test. The results are presented for AUC for ADP test (*A*), AUC in arachidonic acid test (*B*), and for AUC in thrombin receptor activator peptide 6 test (*C*). *$P < 0.05$. AUC, area under the curve; ADP, adenosine-5 diphosphate; COVID-19, coronavirus disease 2019.

Comparing the results from thromboelastometric assays of COVID-19 patients and healthy controls, significant differences were observed in extrinsic activation assay for the clotting time (mean ± SD, 88 ± 22 s *vs.* 60 ± 7 s; 22 [95% CI, 15 to 33]; $P < 0.001$) and the maximum clot firmness (68 ± 5 mm *vs.* 57 ± 4 mm; 11 [95% CI, 8 to 14]; $P < 0.001$).

Further, significant differences were observed in intrinsic activation assay for the clotting time (262 ± 120 s *vs.* 163 ± 12 s; 47 [95% CI, 25 to 92]; $P < 0.001$) and the maximum

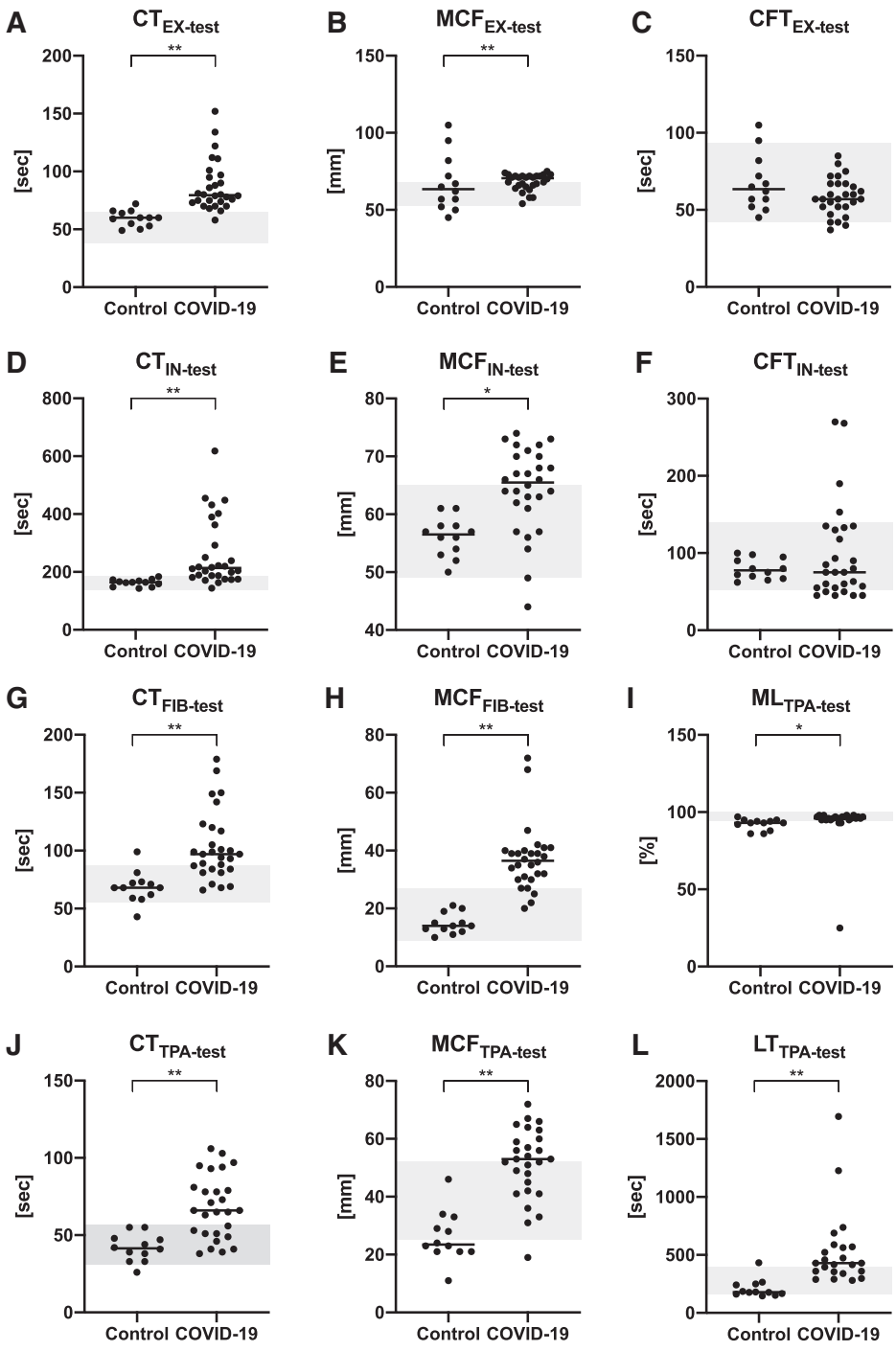

Fig. 2. Results of thromboelastometry in COVID-19–infected patients and healthy controls. *Scatter plots* of thromboelastometry. The *line* represents the median. Data comparisons were made using Mann–Whitney U test. Presented are the results of extrinsic activation assay: (*A*) clotting time extrinsic activation, (*B*) maximum clot formation, extrinsic activation, and (*C*) clot formation time, extrinsic activation; intrinsic activation: (*D*) clotting time intrinsic activation, (*E*) maximum clot formation, intrinsic activation, (*F*) clot formation time, intrinsic activation; contribution of fibrin to clot firmness assay (fibrin test): (*G*) clotting time, contribution of fibrin to clot firmness, (*H*) maximum clot formation, contribution of fibrin to maximum clot firmness; extrinsic activation and activation of fibrinolysis by Tpa (Tpa-test): (*I*) maximum lysis, extrinsic activation and activation of fibrinolysis by Tpa, (*J*) clotting time, extrinsic activation and activation of fibrinolysis by Tpa, (*K*) maximum clot firmness, extrinsic activation and activation of fibrinolysis by Tpa, (*L*): lysis time, extrinsic activation and activation of fibrinolysis by Tpa. $^{*}P < 0.01$; $^{**}P < 0.001$. CFT, clot formation time; COVID-19, coronavirus disease 2019; CT, clotting time; LT, lysis time; MCF, maximum clot firmness; ML, maximum lysis; Tpa, tissue plasminogen activator.

clot firmness (64 ± 8 mm *vs.* 56 ± 3 mm; 9 [95% CI, 5 to 13]; $P = 0.001$).

We also identified differences in the contribution of fibrin to clot firmness assay for the clotting time (104 ± 31 mm *vs.* 69 ± 14 mm; 28 [95% CI, 16 to 46]; $P < 0.001$) and the maximum clot firmness (37 ± 11 mm *vs.* 15 ± 4 mm; 21 [95% CI, 17 to 26]; $P < 0.001$).

Further, significant differences were found in the assay analyzing the extrinsic activation and activation of fibrinolysis by Tpa for the clotting time (68 ± 21 s *vs.* 42 ± 9 s; 25 [95% CI, 12 to 39]; $P < 0.001$), the maximum clot firmness (51 ± 12 mm *vs.* 26 ± 9 mm; 27 [95% CI, 19 to 33]; $P < 0.001$), the maximum lysis (93 ± 15% *vs.* 92 ± 4%; 3 [95% CI, 2 to 5]; $P = 0.001$), and the lysis time (530 ± 327 s *vs.* 211 ± 80 s; 238 [95% CI, 160 to 326]; $P < 0.001$).

Effect sizes (Cohen's d) were calculated for the not significantly different tests and interpreted per Cohen[12] as follows: AUC for arachidonic acid, −0.29/small effect; AUC for thrombin receptor activating peptide, −0.62/medium effect; clot formation time in extrinsic activation, −0.054/medium effect; and clot formation time in intrinsic activation, 0.44/medium effect.

When checking for associations with the potential confounders age and body mass index, these were not significant in any bivariable analyses. Stratified analysis with respect to sex in markers significantly associated with sex (maximum clot firmness in contribution of fibrin to clot firmness assay and maximum clot firmness in assay of extrinsic activation and activation of fibrinolysis by Tpa) did not change the significance of the results.

Discussion

Our prospective, observational study of 27 patients with COVID-19 infection and moderate to severe ARDS revealed greater fibrinolysis resistance as reflected by thromboelastometry and no greater platelet aggregability using impedance aggregometric testing.

Assessment of coagulation factors and conventional coagulation parameters demonstrated elevated D-dimer levels as described previously and typical to COVID-19.[2] Moreover, we identified a PTT within normal ranges; more vWF antigen, factor VIII, and factor IX; and less protein S, indicative of complement pathway activation, acute phase response, and an association with a procoagulant state. Considering the recent findings of endotheliitis[13] in COVID-19 patients, the elevation of vWF levels may mirror endothelial activation or damage. Such elevated vWF levels, as recently described by others,[14,15] may therefore be considered as a surrogate parameter of endothelial dysfunction, supporting the procoagulant imbalance with a potentially higher risk of venous thromboembolism. The elevated factor IX levels cannot be conclusively explained by the data obtained. Since both individual variability[16] and advanced age[17] have been observed in association with higher levels of factor IX, no reliable differentiation can be drawn. Nevertheless, we would like to highlight the proven significance of factor VIII and factor IX elevations in regard to an associated high risk of venous thrombotic events,[18,19] which further highlights the need for a sophisticated anticoagulation regimen in these patients. It remains speculative whether the reported lower levels of factor XIII are acquired; such low levels may result from either an increased consumption or a reduced production and should be addressed in future studies.

In contrast with the findings of Ranucci *et al.*,[20] the fibrinogen values of the COVID-19 patients rarely exceeded but were close to the upper reference range, which contributed to the clinically significantly greater clot strength detected. While we observed a prolonged clotting time in intrinsic activation for patients suffering from COVID-19, this finding may in part have been altered by treatment with unfractionated heparin in eight patients. The observed higher levels of factor VIII and IX suggest that the prolonged clotting time in the intrinsic activation assay is not related to a factor deficiency. However, the lower factor XII level might have contributed to the prolonged clotting time of this test. Further, the results obtained within the assay of extrinsic activation and activation of fibrinolysis by Tpa should not be affected by unfractionated heparin or low-molecular-weight heparin, as it is activated *via* the extrinsic pathway (tissue factor) and contains a heparin antagonist (polybrene).

The analysis of impedance aggregometric assays revealed values within the normal reference ranges. In comparison to healthy controls, mean results of AUC for ADP were significantly lower, which is in line with changes in platelet aggregation in bacterial sepsis.[21] In our patient cohort, we observed thrombocytosis in the majority of cases, in contrast to recently published data describing thrombocytopenia as a common finding in patients with severe COVID-19 infection.[22,23] Given that the analyses in this study were carried out on a single occasion, the most likely cause is reactive thrombocytosis, which has been reported for COVID-19. Impedance aggregometric measurements revealed no greater platelet aggregation, although it might have been suspected given the increased reports of thromboembolic events.[4,24] However, definitive conclusions on the platelet function in patients suffering from COVID-19 may not be drawn from the analysis presented within this manuscript, as sophisticated analyses including receiver operating characteristics curves and serial measurement during the hospital stay from a bigger patient population are warranted to further substantiate the current findings.

The thromboelastometric results of our study using the ClotPro reinforce and complement the current concept of a hypercoagulable pattern in COVID-19 patients with a profound derangement of hemostasis.[20] Hence, the performance of the recently introduced assay of extrinsic activation and activation of fibrinolysis by Tpa revealed new and relevant results.[25] Using the same reagents as in the extrinsic

activation assay, this test is based on additional recombinant Tpa to induce fibrinolysis. Thus, the presence of lysis inhibitors (*e.g.*, tranexamic acid) and their influence on blood clotting as well as Tpa-induced lysis can be assessed. Our results of the lysis time of this test indicate that there is a greater fibrinolysis resistance in COVID-19 patients in comparison to healthy controls.

In addition to the reported elevations in serum D-dimer levels for COVID-19 reflecting an activation of the coagulation system consistent with the described various clinical thrombotic events in these patients,[4,24,26] our results of the assay of extrinsic activation and activation of fibrinolysis by Tpa might therefore reflect a greater fibrinolysis resistance, which reinforces the procoagulant state described and complements the results of recently published viscoelastic measurements.[14,27] Of note, hypofibrinolysis or fibrinolysis shutdown is characterized by decreased fibrinolysis in assays without Tpa challenge.[21,28–30]

Moreover, the findings of our analyses are consistent with emerging observations suggesting that COVID-19 has features distinct from typical ARDS. In addition to the considerably well-preserved lung mechanics despite a severe hypoxemia, as characterized by high respiratory compliance, high shunt fraction, and prolonged mechanical ventilation,[31] Magro *et al.* reported an association with the involvement of complement components within the pulmonary septal microvasculature, which is atypical for classic ARDS.[32] Such extensive complement involvement may lead to complex-mediated microvascular endothelial cell injury and subsequent activation of the coagulation pathway, which might explain our findings.[33] In addition, recently published findings of direct viral infection of endothelial cells and diffuse endothelial inflammation leading to endothelial dysfunction might further contribute to the observed procoagulant state of hemostasis.[13]

Dysregulated fibrinolysis, often observed in the context of critical illness,[34,35] can lead to a so-called "fibrinolytic shutdown" as a result of various imbalances of the hemostaseological homeostasis.[36,37] The elevated D-dimer levels in combination with the elevated fibrinogen concentrations and the results of the viscoelastic analysis may indicate a greater fibrinolysis resistance, an interpretation that is consistent with recently published research.[30,38] These alterations could contribute to the observed laboratory changes consistent with disseminated intravascular coagulation, but distinctively lead to thromboembolic events in these patients.

Further investigations and the determination of parameters representing the complex system of fibrinolysis (*e.g.*, plasmin, plasminogen activator inhibitor, or thrombin-activated fibrinolysis inhibitor) might provide further insights into this topic. To gain definitive answers about the pathophysiology of coagulation in COVID-19, data from a larger patient population are warranted.

Study Limitations

The major threat to internal validity results from a sampling bias of the study population: for the COVID-19 cohort, we included a substantial number of patients referred to our hospital by primary care providers. This may have resulted in an assessment of patients more severely affected by COVID-19 compared to the general COVID-19 population. This sampling bias is further substantiated by the fact that, in contrast to the overall COVID-19 population, all COVID-19 patients of this study were mechanically ventilated. The analysis of these highly selected, severely affected patients may result in an overestimation of the results observed within our study. Differential misclassification may result from the sedation/intubation of COVID-19 patients, leading to uncertainties regarding the patient history when compared to fully awake, healthy controls. We have demonstrated significant differences between both healthy controls and the COVID-19 cohort, which might serve as confounding variables and potentially interfere with the independent variable (diagnosis of COVID-19). However, little is known about the effects of this novel disease, and matching of a cohort by age, body mass index, and renal replacement therapy would both fail to reflect the complexity of this disease and leave other confounders unaddressed (such as diabetes, among others). Our stratified or bivariable analysis showed only minor differences or no influence of the confounders sex, age, and body mass index on the analyzed coagulation alterations from point-of-care diagnostics. At this point, we sought to provide a comparison to a healthy control group with all the limitations inferred by such a comparison, but we carefully avoid overclaiming our findings. However, we would like to stress that some studies on rotational thromboelastometry[39,40] have shown significant changes for a higher age toward hypercoagulability, and therefore, the age difference between the groups may have influenced our results. Moreover, an influence of obesity[41,42] and diabetes mellitus[43–45] has also been previously described and may have affected our results reported. Due to the novelty of the assay, no formal analysis of measures of reliability or validity has been published for the ClotPro yet. To avoid confusion regarding the maximum lysis results, which might appear contradictory to the lysis time results, it has to be considered that for technical reasons, the lowest amplitude of the maximum lysis is 1.5 mm during lysis, and therefore, the maximum lysis cannot reach 100%.

While we consider the internal validity of this study to be high regarding the values obtained by both laboratory testing and thromboelastometry, limitations arise from the nonlongitudinal nature of the measurements obtained for this study. Moreover, we assume that a sequential analysis of platelet function may better characterize alterations than a single analysis as performed in our study, since platelet function is a very dynamic process and blood sampling for impedance aggregometry was done in (mean) 7 ± 3.5 days after ICU admission. Therefore, increased platelet

aggregation at an early phase of COVID-19 with subsequent exhaustion of platelets cannot be excluded by this study—particularly since the AUC of the ADP test was significantly decreased compared to the control group. While we provide a detailed analysis of the fibrinolysis resistance at one point of the disease, future studies should provide longitudinal information on the time point associated with the named pathology. We hypothesize that the findings of our study might be generalizable to the general COVID-19 population with the limitation that our findings were obtained in a group of severely affected, ventilated patients. Other manuscripts have been published on point-of-care diagnostics in COVID-19 demonstrating results similar to the findings of our study. However, future studies repeating measurements on thromboelastometry in patients with COVID-19 are warranted to replicate our findings and to further improve this study's external validity.

Conclusions

Although critically ill patients with COVID-19 have a hypercoagulable state, we did not find greater platelet aggregability based on impedance aggregometric testing. Moreover, thromboelastometry in our patients revealed greater fibrinolysis resistance. These findings may contribute to the understanding of the hypercoagulable state in critically ill patients with COVID-19 and be used to further develop appropriate anticoagulation regimens for the prevention and treatment of thromboembolic events.

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Competing Interests

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