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

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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 viscoelastic (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 receiver operating characteristics curve [AUC] in adenosine diphosphate test, 68 ± 37 U vs. 91 ± 29 U [Hodges–Lehmann 95% CI, −27 (−48 to −1); P = 0.043]; AUC in arachidonic acid test, 102 ± 54 U vs. 115 ± 26 U [Hodges–Lehmann 95% CI, −21 (−51 to 21); P = 0.374]; AUC in thrombin receptor activating peptide 6 test, 114 ± 61 U vs. 144 ± 31 U [Hodges–Lehmann 95% CI, −31 (−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 [Hodges–Lehmann 95% CI, 21 (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 [Hodges–Lehmann 95% CI, 238 (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.

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 viscoelastic 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.

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 COVID-19–related coagulation changes using aggregometric (Multiplate; Roche Diagnostics) and...
viscoelastic (ClotPro; enicor GmbH) 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/m²) 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 twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day. In the case of vasopressor therapy, the regimen was changed to administration of unfractionated heparin twice a day.

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 A₂; 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 viscoelastic measurements of whole blood coagulation by recording kinetic changes in a sample of citrated whole blood, similar to 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 fac-

tor–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 plate-

let aggregation, which evaluates the contribution of fibrin
to clot firmness; and tissue factor–triggered extrinsic path-

way 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 ampli-

tude 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 nor-
mality using the Kolmogorov–Smirnov test. Data compar-
isons 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 calcu-
lations/analyses were performed with SPSS (Version 25),

R version 3.6, and GraphPad Prism (Version 8). 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/m². The mean age was 60 ± 13 yr. The mean

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/m² (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 |         |
| Pao2/Fio2 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; Fio2, fraction of inspired oxygen; Pao2, partial pressure arterial oxygen.
ICU stay from admission until impedance aggregometric and viscoelastic assessment was 7 ± 3.5 days. The most frequent pre-existing 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 42.14 The control group consisted of 12 healthy volunteers without any known pre-existing conditions. The mean age of the control group was 38 ± 6 years; the mean body mass index was 24.5 ± 2.6 kg/m², and there were statistically significant differences in age (P < 0.0001), body mass index (P < 0.0001), prevalence of pre-existing 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 PT, 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 IE (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 ± 107.9%), and lesser activity of factor XIII (64.5 ± 35.1%) and protein S (45 ± 30.1%). Thrombocytosis (defined as platelet count greater than 350 10⁹/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.

**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.18 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; 95% CI, −31 [−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.
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, 88 ± 22 s vs. 60 ± 7 s; 95% CI, 22 [15 to 33]; P < 0.001) and the maximum clot firmness (mean, 68 ± 5 mm vs. 67 ± 4 mm; 95% CI, 11 (8–14); P < 0.001).

Further, significant differences were observed in intrinsic activation assay for the clotting time (mean, 262 ± 120 s vs. 163 ± 12 s; 95% CI, 47 (25–92); P < 0.001) and the maximum clot firmness, mm (mean, 64 ± 8 vs. 56 ± 3; 95% CI, 9 (5–13); P < 0.001).

Table 3. Results of Impedance Aggregometric and Thromboelastometric Assays

|                          | COVID-19 (n = 27) | Healthy Controls (n = 12) | 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                  | 6 (−17 to 5)                             | 0.265  |
| Maximum clot firmness, mm| 68 ± 5           | 57 ± 4                   | 11 (9–14)                                | <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 reference ranges of AUC are highlighted by a gray area. One patient was excluded due to thrombopenia less than 70 × 10^3/μl. Eight patients on therapy with acetylsalicylic acid were excluded from analysis of AUC for arachidonic acid. Data comparisons were made using Mann–Whitney U test. The results are presented for AUC for ADP (A), AUC for arachidonic acid (B), and AUC for thrombin receptor activator peptide 6 (C). *P < 0.05. AUC, area under the curve; ADP, adenosine-5 diphosphate; COVID-19, coronavirus disease 2019.
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: (A) clotting time extrinsic test, (B) maximum clot firmness extrinsic test, and (C) clot formation time extrinsic test; intrinsic activation: (D) clotting time intrinsic test, (E) maximum clot firmness intrinsic test, and (F) clot formation time intrinsic test; contribution of fibrin to clot firmness: (G) clotting time fibrin test; contribution of fibrin to maximum clot firmness: (H) maximum clot firmness fibrin test; extrinsic activation and activation of fibrinolysis by Tpa: (I) maximum lysis Tpa test, (J) clotting time Tpa test, (K) clot formation time Tpa test, and (L) maximum clot firmness Tpa test. *P < 0.01; **P < 0.001. COVID-19, coronavirus disease 2019; Tpa, tissue plasminogen activator.
maximum clot firmness (mean, 64 ± 8 mm vs. 56 ± 3 mm; 95% CI, 9 [5 to 13]; \( P = 0.001 \)).

We also identified differences in the contribution of fibrin to clot firmness for the clotting time (mean, 104 ± 31 s vs. 69 ± 14 mm; 95% CI, 28 [16 to 46]; \( P < 0.001 \)) and the maximum clot firmness (mean, 37 ± 11 mm vs. 15 ± 4 mm; 95% CI, 21 [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 (mean, 68 ± 21 s vs. 42 ± 9 s; 95% CI, 25 [12 to 39]; \( P < 0.001 \)), the maximum clot firmness (mean, 51 ± 12 mm vs. 26 ± 9 mm; 95% CI, 27 [19 to 33]; \( P < 0.001 \)), the maximum lysis (mean, 93 ± 15% vs. 92 ± 4%; 95% CI, 3 [2 to 5]; \( P = 0.001 \)), and the lysis time (mean, 530 ± 327 s vs. 211 ± 80 s; 95% CI, 238 [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 bivariate 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 endothelitis\(^{13} \) 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.\(^{25} \) Hence, the performance of the recently introduced assay of extrinsic activation and activation of fibrinolysis by Tpa revealed new and
relevant results. 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, 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. Of note, hypofibrinolysis or fibrinolysis shutdown characterized by decreased fibrinolysis in assays without Tpa challenge.

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, Magro et al. reported an association with the involvement of complement components within the pulmonary septal microvasculature, which is atypical for classic ARDS. 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. 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.

Dysregulated fibrinolysis, often observed in the context of critical illness, can lead to a so-called “fibrinolytic shutdown” as a result of various imbalances of the hemostological homeostasis. 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. 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 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 and diabetes mellitus 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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References

1. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, Liu L, Shan H, Lei C-h, Hui DSC, Du B, Li L-j, Zeng G, Yu P, Yang B, Wang X-l, Xiang M, Xing Y, Li G, He J, Liu J, Nie Z, Zhong N, Wu Y, Chen L, Cao J, Huang C, Wang Y, Li X, Guan H, Wei Y, Zhu H, MacKeith J, Jiang L, Xia D, Wei X, Liu N, Yan Z, Su S, Zhang D, Liu X, Xiao J, Wu J, Wang X, Zhou S, Gong F, Yu F, Liu Y, Jiang X, Chen Z, Li J, Yang Y, Yan Y, Wang Y, He J, Li S, Hui D, Xu X, Gu X, Yu Y, Zhou L, Yu Z, Zhang L, Lu R, Zhao X, Xiong J, Liu J, Lei W, Zhang Y, Yang G, Wei W, Wu X, Xing Y, Wang X, Chu T, Chen L, Cao L, Wei W, Gao X, Zhao W, Xu X, Li H, Miao Q, Wang X, Gao J, Zhang Y, Yang H, Kong L, Jiang X, Wang T, Chen M, Yu T, Li X, Li L, Wang Z, Shang X, Wu H, Wang X, Zhou F, Li X, Gu J, Wang W, Wang Y, Wang Y, Liao Y, Zhou J, Han Y, Wang Y, Wang S, Li H, Wang D, Yang H, Zhang X, Cai J, Zhao Y, Exadaktylos A, Hui D, Yu T, Song C, Liu Y, Liu L, Wang P, Cai X, Wu J, Xia J, Gao W, Han Y, Liu Y, Gu H, Li T, Wang M, Liu Y, Li M, Chen J, He J, Liu X, Qian W, Chen W, Wang X, Zheng X, Gao X, Liu S, Hu L, Wang H, Zhao W, Wang Y, Wang X, Jiang L, Wang Y, Wang X, Zhang Y, Zhao H, Sun R, Han L, Zhang Q, Wu J, Zang Y, Pan Z, Liu Z, Meng Y, Zhang Y, Zheng Q, Gao J, Wang X, Sun W, Wang Y

2. Han H, Yang L, Liu R, Liu F, Wu K-j, Li J, Liu X-h, Zhu C-l: Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. 2020; doi: 10.1111/bjh.16725 [Epub ahead of print]

3. Tang N, Li D, Wang X, Sun Z: Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020; 18:844–7

4. Klok F, Kruip M, van der Meer N, Arbous M, Gommers D, Kant K, Kaptein F, van Paassen J, Stals M, Huisman M: Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020; 191:145–7

5. Kollia GS, Syrigos K: Thromboembolic risk and anticoagulant therapy in COVID-19 patients: Emerging evidence and call for action. Br J Haematol2020; 191:145–7

6. Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, Merouani K: High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost 2020; 18:1743–6

7. Poston JT, Patel BK, Davis AM: Management of critically ill adults with COVID-19. JAMA 2020; 323:1839–41

8. Adam EH, Baro D, Schmidt P, Mutlak H, Zacharowski K, Hanke AA, Weber CF: Aggregometric assessment of
clonidine’s impact on the efficacy of dual platelet inhibition. Clin Lab 2014; 60:1533–9
9. Multiplate Analyzer [package insert]. Basel, Switzerland: Roche Diagnostics; 2013
10. Le Gall JR, Lemeshow S, Saulnier F: A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993; 270:2957–63
11. Hanke AA, Roberg K, Monaca E, Sellmann T, Weber CF, Rahe-Meyer N, Görlinger K: Impact of platelet count on results obtained from multiple electrode platelet aggregometry (Multiplate). Eur J Med Res 2010; 15:214–9
12. Cohen J: The effect size, Statistical Power Analysis for the Behavioral Sciences, 2nd ed. Hillsdale, NJ, Lawrence Erlbaum 1988, pp 77–83
13. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H: Endothelial cell infection and endothelitis in COVID-19. Lancet 2020; 395:1417–8
14. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, Pesenti A, Peyvandi F, Tripodi A: Hypercoagulability of COVID-19 patients in intensive care unit. A report of thromboelastography findings and other parameters of hemostasis. J Thromb Haemost 2020; 18:1738–42
15. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, Merdji H, Clerc-Ishii R, Schnick M, Fagot Gandet F: High risk of thrombosis in patients with severe SARS-CoV-2 infection: A multicenter prospective cohort study. Intensive Care Med 2020; 46:1089–98
16. Reiner A, Davie E: The physiology and biochemistry of factor IX. Haemostasis and Thrombosis, 3rd edition. Edinburgh, Churchill Livingstone, 1994, pp 309–324
17. Sweeney JD, Hoeping LA: Age-dependent effect on the level of factor IX. Am J Clin Pathol 1993; 99:687–8
18. Heikal NM, Murphy KK, Crist RA, Wilson AR, Rodgers GM, Smock KJ: Elevated factor IX activity is associated with an increased odds ratio for both arterial and venous thrombotic events. Am J Clin Pathol 2013; 140:680–5
19. Jenkins PV, Rawley O, Smith OP, O'Donnell JS: Elevated factor VIII levels and risk of venous thrombosis. Br J Haematol 2012; 157:653–63
20. Ranucci M, Ballotta A, Di Dedda U, Bayshnikova E, Dei Poli M, Resta M, Falco M, Albano G, Menicanti L: The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. J Thromb Haemost 2020; 18:1747–51
21. Adamzik M, Eggmann M, Frey UH, Görlinger K, Bröcker-Preuss M, Marggraf G, Säner F, Eggebrecht H, Peters J, Hartmann M: Comparison of thromboelastometry with procalcitonin, interleukin 6, and C-reactive protein as diagnostic tests for severe sepsis in critically ill adults. Crit Care 2010; 14:R178
22. Xu P, Zhou Q, Xu J: Mechanism of thrombocytopenia in COVID-19 patients. Ann Hematol 2020; 99:1205–8
23. Lippi G, Plebani M, Henry BM: Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. Clin Chim Acta 2020; 506:145–48
24. Cui S, Chen S, Li X, Liu S, Wang F: Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J Thromb Haemost. 2020; 18:1421–24
25. Wang J, Hajizadeh N, Moore EE, McIntyre RC, Moore PK, Veress LA, Yaffe MB, Moore HB, Barrett CD: Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A case series. J Thromb Haemost 2020; 18:1752–55
26. Chen J, Wang X, Zhang S, Liu B, Wu X, Wang Y, Wang X, Yang M, Sun J, Xie Y: Findings of acute pulmonary embolism in COVID-19 patients. SSRN 2020; doi: 10.2139/ssrn.3548771
27. Spiezia L, Boscolo A, Polletto F, Cerruti L, Tiberio I, Campello E, Navalesi P, Simioni P: COVID-19-related severe hypercoagulability in patients admitted to intensive care unit for acute respiratory failure. Thromb Haemost 2020; 120:998–1000
28. Schmitt FCF, Manolov V, Morgenstern J, Fleming T, Heitmeier S, Uhle F, Al-Saeedi M, Hackert T, Bruckner T, Schöchli H, Weigand MA, Hofer S, Brenner T: Acute fibrinolysis shutdown occurs early in septic shock and is associated with increased morbidity and mortality: Results of an observational pilot study. Ann Intensive Care 2019; 9:19
29. Stettler GR, Moore EE, Moore HB, Nunns GR, Silliman CC, Banerjee A, Saaua A: Redefining post-injury fibrinolysis phenotypes using two viscoelastic assays. J Trauma Acute Care Surg 2019; 86:679–85
30. Wright FL, Vogler TO, Moore EE, Moore HB, Wohlauer MV, Urban S, Nydami TL, Moore PK, McIntyre RC Jr: Fibrinolysis shutdown correlation with thromboembolic events in severe COVID-19 infection. J Am Coll Surg 2020; 231:193–203, e1
31. Gattinoni L, Coppola S, Cressoni M, Busana M, Chiumello D: Covid-19 does not lead to a “typical” acute respiratory distress syndrome. Am J Respir Crit Care Med 2020; 201:1299–1300
32. Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, Baxter-Stoltzfus A, Laurence J: Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. Transl Res 2020; 220:1–13
33. Vallet B, Wiel E: Endothelial cell dysfunction and coagulation. Crit Care Med 2001; 29(suppl):S36–41
34. Toh CH, Dennis M: Disseminated intravascular coagulation: Old disease, new hope. BMJ 2003; 327:974–7
35. Chang JC: Disseminated intravascular coagulation: Is it fact or fancy? Blood Coagul Fibrinolysis 2018; 29:330–7
36. Gando S, Wada T: Disseminated intravascular coagulation in cardiac arrest and resuscitation. J Thromb Haemost 2019; 17:1205–16
37. Moore HB, Moore EE, Huebner BR, Dzieciatkowska M, Stettler GR, Nunns GR, Lawson PJ, Ghasabyan A, Chandler J, Banerjee A, Silliman C, Sauaia A, Hansen KC: Fibrinolysis shutdown is associated with a fivefold increase in mortality in trauma patients lacking hypersensitivity to tissue plasminogen activator. J Trauma Acute Care Surg 2017; 83:1014–22
38. Zátroch I, Smudla A, Babik B, Tánzos K, Kóbóri L, Szabó Z, Fazakas J: Procoagulation, hypercoagulation and fibrinolytic “shut down” detected with ClotPro® viscoelastic tests in COVID-19 patients. Orv Hetil 2020; 161:899–907
39. Lang T, Bauters A, Braun SL, Pötzsch B, von Pape KW, Kolde HJ, Lakner M: Multi-centre investigation on reference ranges for ROTEM thromboelastometry. Blood Coagul Fibrinolysis 2005; 16:301–10
40. Schenk B, Görlinger K, Trembl B, Tauber H, Fries D, Niederwanger C, Oswald E, Bachler M: A comparison of the new ROTEM® sigma with its predecessor, the ROTEMdelta. Anaesthesia 2019; 74:348–56
41. Campello E, Spiezia L, Zabeo E, Maggiolo S, Vettor R, Simioni P: Hypercoagulability detected by whole blood thromboelastometry (ROTEM®) and impedance aggregometry (MULTIPATE®) in obese patients. Thromb Res 2015; 135:548–53
42. Taura P, Rivas E, Martinez-Pallé G, Blasi A, Holguera JC, Balust J, Delgado S, Lacy AM: Clinical markers of the hypercoagulable state by rotational thrombelastometry in obese patients submitted to bariatric surgery. Surg Endosc 2014; 28:543–51
43. Binay C, Bozkurt Turhan A, Simek E, Bor O, Akay OM: Evaluation of coagulation profile in children with type 1 diabetes mellitus using rotational thromboelastometry. Indian J Hematol Blood Transfus 2017; 33:574–80
44. Feuring M, Wehling M, Burkhardt H, Schultz A: Coagulation status in coronary artery disease patients with type II diabetes mellitus compared with non-diabetic coronary artery disease patients using the PFA-100® and ROTEM®. Platelets 2010; 21:616–22
45. Yürekli BP, Ozcebe OI, Kirazli S, Gürlek A: Global assessment of the coagulation status in type 2 diabetes mellitus using rotation thromboelastography. Blood Coagul Fibrinolysis 2006; 17:545–9