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Thromboelastography clot strength profiles and effect of systemic anticoagulation in COVID-19 acute respiratory distress syndrome: a prospective, observational study

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Abstract. – OBJECTIVE: Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) infection may yield a hypercoagulable state with fibrinolysis impairment. We conducted a single-center observational study with the aim of analyzing the coagulation patterns of intensive care unit (ICU) COVID-19 patients with both standard laboratory and viscoelastic tests. The presence of coagulopathy at the onset of the infection and after seven days of systemic anticoagulant therapy was investigated.

PATIENTS AND METHODS: Forty consecutive SARS-CoV-2 patients, admitted to the ICU of a University hospital in Italy between 29th February and 30th March 2020 were enrolled in the study, providing they fulfilled the acute respiratory distress syndrome criteria. They received full-dose anticoagulation, including Enoxaparin 0.5 mg·kg-1 subcutaneously twice a day, unfractionated Heparin 7500 units subcutaneously three times daily, or low-intensity Heparin infusion. Thromboelastographic (TEG) and laboratory parameters were measured at admission and after seven days.

RESULTS: At baseline, patients showed elevated fibrinogen activity [rTEG-Ang 80.5° (78.7 to 81.5); TEG-ACT 78.5 sec (69.2 to 87.9)] and an increase in the maximum amplitude of clot strength [FF-MA 42.2 mm (30.9 to 49.2)]. No alterations in time of the enzymatic phase of coagulation [CKH-K and CKH-R, 1.1 min (0.85 to 1.3) and 6.6 min (5.2 to 7.5), respectively] were observed. Absent lysis of the clot at 30 minutes (LY30) was observed in all the studied population. Standard coagulation parameters were within the physiological range: [INR 1.09 (1.01 to 1.20), aPTT 34.5 sec (29.7 to 42.2), antithrombin 97.5% (89.5 to 115)]. However, plasma fibrinogen [512.5 mg·dl-1 (303.5 to 605)], and D-dimer levels [1752.5 ng·ml-1 (698.5 to 4434.5)], were persistently increased above the reference range. After seven days of full-dose anticoagulation, average TEG parameters were not different from baseline (rTEG-Ang $p = 0.13$, TEG-ACT $p = 0.58$, FF-MA $p = 0.24$, CK-R $p = 0.19$, CKH-R $p = 0.35$), and a persistent increase in white blood cell count, platelet count and D-dimer was observed (white blood cell count $p < 0.01$, neutrophil count $p = 0.02$, lymphocyte count $p < 0.01$, platelet count $p = 0.13 < 0.01$, D-dimer levels $p = 0.02$).

CONCLUSIONS: SARS-CoV-2 patients with acute respiratory distress syndrome show elevated fibrinogen activity, high D-dimer levels and maximum amplitude of clot strength. Platelet count, fibrinogen, and standard coagulation tests do not indicate a disseminated intravascular coagulation. At seven days, thromboelastographic abnormalities persist despite full-dose anticoagulation.

Key Words:
Coronavirus, Respiratory insufficiency, Hemostasis, Blood coagulation, Critical care, Pneumonia, Thrombosis, Infection, Critical illness.

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Abbreviations

SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus-2; ARDS, Acute respiratory distress syndrome; DVT, Deep Venous Thrombosis; COVID-19, Coronavirus Disease 19; VTE, Venous Thromboembolism; aPTT, Activated Partial Thromboplastin Time; PT, prothrombin time; INR, International Normalized Ratio; TEG, Thromboelastography; SOFA, Sequential Organ Failure Assessment score; DIC, Disseminated Intravascular Coagulation; BMI, Body Mass Index; ROTEM, Rotational Thromboelastometry; LMWH, Low Molecular Weight Heparin; UFH, Unfractionated Heparin; PCR, Polymerase Chain Reaction; IRB, Institutional Review Board; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; ISTH, International Society on Thrombosis and Hemostasis; ICU, Intensive Care Unit; CK, Kaolin TEG; CKH, Kaolin TEG with heparinase; rTEG, Rapid TEG; CFF, TEG functional fibrinogen; R, Time to Fibrin Formation; K, angle constant; α, angle; MA, maximal clot strength; LY30, percentage decrease in amplitude at 30 minutes post-MA; KDE, Kernel Density Estimate; IQR, Interquartile Range; SD, Standard Deviation; WBC, White Blood Cell Count; TF, Tissue Factor

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) can cause severe interstitial lung disease and acute respiratory distress syndrome (ARDS), needing ventilatory support and admission to the intensive care unit[1,2]. Microvascular thrombosis is a hallmark of ARDS, and patients with SARS-CoV-2 commonly show overproduction of early response pro-inflammatory cytokines, hypercoagulation, and markedly elevated D-dimer levels[3]. Although these findings are associated with poor prognosis, data suggest that anticoagulant therapy may be beneficial, as observed in patients with sepsis[4-10]. Klok et al[11] evaluated the incidence of the composite outcome of symptomatic acute pulmonary embolism, deep-vein thrombosis (DVT), ischemic stroke, myocardial infarction or systemic arterial embolism in 184 SARS-CoV-2 positive patients admitted to the ICU. They found thrombotic complications occurred in 31% of those receiving standard venous thromboembolism (VTE) prophylaxis. In a multicenter retrospective analysis of 187 patients, Shah et al[12] found an incidence of 43.3% of pulmonary embolism and DVP, and 13.3% of arterial thrombotic events. Maatman et al[13] observational analysis on 240 SARS-CoV-2 positive patients hypothesized that the standard VTE prophylaxis might be inadequate in preventing thrombotic complications in severe Coronavirus Disease 19 (COVID-19). A typical feature of COVID-19 syndrome is a severe impairment of the fibrinolytic process with an increase in D-dimer levels, associated with an increase in thromboembolic events[14]. Standard coagulation tests, such as activated partial thromboplastin time (aPTT), and prothrombin time (PT), are not useful in identifying hypercoagulability, and do not explore platelet function and fibrinolysis. In addition, fibrinogen levels and absolute platelet count provide no information about their functionality.

Viscoelastic whole blood tests may detect coagulation abnormalities that are not detectable by conventional tests[15-17]. Thromboelastography (TEG) provides useful information about hypercoagulability, assesses clot lysis, and disseminated intravascular coagulation (DIC)[18]. Observational studies[12,14,19-27] involving small cohorts have shown that thromboelastography is reliable in identifying hypercoagulability and fibrinolysis shutdown in COVID-19 patients.

TEG®6s (Haemonetics, Braintree, MA, USA), a new generation of TEG devices, was introduced recently[28-31]. Lloyd-Donald et al[32] demonstrated in a small group of critically ill patients that TEG®6s generates similar results to the TEG®5000 model.

Although several studies have already investigated the coagulation assessment of COVID-19 patients, it is still unclear how coagulation changes can affect or reflect the course of the COVID-19 disease.

We conducted a prospective observational study to assess the effects of seven-day full-dose systemic anticoagulation on coagulation parameters investigated by standard and thromboelastographic methods in a cohort of patients with ARDS due to COVID-19.

Patients and Methods

In this single-center prospective observational study, consecutive patients who were admitted to ICU with respiratory failure and with a confirmed molecular diagnosis (a positive real time polymerase chain reaction for viral RNA) of COVID-19 *via* an upper or lower respiratory tract specimen were considered for enrolment in this study. Patients admitted between 29th February-30th March 2020 at a University hospital in Italy were included provided they fulfilled the ARDS criteria[33], received full-dose anticoagulation (Enoxaparin 0.5 mg·kg^{-1} subcutaneously twice a day, unfractionated heparin 7500 units subcutaneously three times daily, or low-inten-

sity heparin infusion). Patients with one or more of the following criteria were excluded: history of cirrhosis; preexistent coagulation or hemostasis disorders; active malignancy; Body Mass Index (BMI) < 20; supplemental steroid therapy; thrombocytopenia with a platelet count less than $150x10^9 \cdot l^{-1}$; anti-platelet medications or vitamin K agonists at the time of ICU admission.

Ethical approval for this study was provided by the local Institutional Review Board, and informed consent was obtained according to committee recommendations. This study is reported following the reporting guidelines for a cohort study by Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).

Procedures

Within the first 24 hours of ICU admission, demographic and clinical data were recorded, and blood samples for routine standard hematological parameters were obtained in vacutainer tubes from an arterial line. Two blood citrate samples (3 ml, sodium citrate solution, Vacuette® Blood Tubes) were also collected for the laboratory standard coagulation tests (aPTT, INR, D-dimer levels, Antithrombin) and TEG®6s assessment.

DIC score per International Society on Thrombosis and Haemostasis (ISTH) criteria[34,35], and Sequential Organ Failure Assessment (SOFA)[36] score, were calculated for each patient on admission to ICU.

All laboratory tests were repeated seven days after initial analysis, unless patients were discharged or deceased.

TEG®6s assays consisted in: Kaolin TEG (CK), which describes the intrinsic pathway activation and can assess the overall coagulation function; Kaolin TEG with heparinase (CKH) which neutralizes the effects of heparin and can assess the presence of heparin or heparinoids; Rapid TEG (rTEG), which describes both the intrinsic and extrinsic pathway using both kaolin and tissue factor (TF); TEG functional fibrinogen (CFF), which uses TF as a coagulation activator and GpIIb/IIIa inhibitors to neutralize platelet function in order to measure the fibrinogen contribution to clot formation.

The TEG parameters include reaction time (R, time to fibrin formation, represents the initiation phase and measures the time of latency from the start of the test to initial fibrin formation), clot formation time (K, represents amplification phase and measures the time taken to achieve 20 mm of clot strength), angle or α (K angle, represents the propagation phase and measures the rate of clot formation), maximum amplitude (MA, represents the overall stability of the clot), and amplitude at 30 minutes (LY30, represents the fibrinolysis phase and measures the percentage decrease in amplitude at 30 minutes post-MA).

Based on the increasing evidence[11-13,37,38] of a hypercoagulable state and a very high incidence of thromboembolic events in COVID-19 patients, our institutional protocols advise initiation of full-dose systemic anticoagulation at admission to ICU (Enoxaparin 0.5 $mg \cdot kg^{-1}$ subcutaneous twice daily, unfractionated heparin 7500 units subcutaneously three times daily, or low-intensity heparin infusion). Dosages and duration of anticoagulant therapy were recorded.

Statistical Analysis

Normal distribution of continuous variables was assessed by the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Continuous variables with normal distribution were presented as mean ± standard deviation (SD) and were compared using Student's *t*-test; continuous variables with non-normal distribution were presented as median (Interquartile range, IQR) and were compared by the non-parametric Mann-Whitney test. Categorical variables were presented as numbers (percentages) and were compared across groups using the χ^2 or Fisher's exact test, as appropriate. A two-sided p-value < 0.05 was considered statistically significant in the final analysis. The analysis of the results of the TEG®6s traces was performed, and the distribution density of data was analyzed using the Kernel Density Estimate (KDE). Data comparing differences in TEG®6s parameters after seven days are presented as box plots. Statistical analysis was performed using Stata/IC 16.0.

The majority of studies adopt TEG®5000 or ROTEM, resulting in insufficient data available on results obtained from TEG®6s in SARS-CoV-2 infection. Although the sample size was not calculated, our minimal study sample size per group is sufficient for a pilot study. Julius[39] confirms that a minimum of 12 subjects per group is sufficient for a pilot study.

Results

Forty consecutive COVID-19 patients with ARDS were enrolled in the study. Clinical and laboratory baseline characteristics of the study

population are shown in Table I. The median age was 67.5 (IQR 55 to 77) years old, and 72.5% of patients were male. The mean SOFA score was 5±2.9, and mean DIC score was 2.9±0.6. Half (52.5%) of enrolled patients had a coexistent cardiovascular disease, and 15% a respiratory disease. Orotracheal intubation was required for 72.5% of patients, and median P_aO_2/F_iO_2 ratio at admission was 190 (IQR 149.5 to 221.5), which identify a moderate ARDS per Berlin criteria[32]. All patients received full-dose systemic anticoagulation with Enoxaparin or unfractionated Heparin. All patients had a TEG®6s assessment at admission, and we could perform a second assessment at 7 days in 26 patients.

The patients were subsequently stratified based on the outcome at 28 days after admission to ICU. Patients with the worst outcome were older [77 years old (IQR 75 to 80) *vs.* 57 years old (IQR 48 to 67), $p < 0.01$], had more cardiovascular [14 (82.4%) *vs.* 7 (30.4%), $p < 0.01$] and respiratory comorbidities [6 (35.3%) *vs.* 0 (0%), $p < 0.01$], had lower S_pO_2 on ICU admission [96% (IQR 95 to 98) *vs.* 98% (IQR 97 to 99), $p < 0.01$], and higher levels of D-dimer [3762 ng·ml^{-1} (IQR 1464 to 6045) *vs.* 851 ng·ml^{-1} (IQR 530 to 2714), $p < 0.01$]. Mean SOFA (7±2.4 *vs.* 4±2.7, $p < 0.01$) and DIC (3.2±0.4 *vs.* 2.7±0.7, $p < 0.01$) scores at 28 days were significantly higher in those patients that died. DIC score in all patients constantly remained less than five, thus suggesting that an overt DIC did not develop in our cohort[34,35]. We found two cases of pulmonary embolism, and one of these patients died within the first 28 days after admission to ICU.

Standard Coagulation Tests

The platelet count, aPTT and INR have not shown any coagulation abnormality in our population [193.5x10^9·l^{-1} (IQR 163 to 281), aPTT 34.5 sec (IQR 29.7 to 42.2), 1.09 (IQR 1.01 to 1.20)] (Table I). D-dimer levels were increased in the study population, with a median value of 1752.5 ng·ml^{-1} (IQR 698.5 to 4434.5).

Thromboelastography

The TEG®6s traces of 40 consecutive patients were analyzed. Table II describes the percentage of deviation from the normal range for each value in our studied cohort of patients at the moment of ICU admission. The second TEG®6s determination, performed after 7 days of anticoagulant treatment, was available for 26 patients (Table III). On admission to ICU, the distribution of CK-R and CKH-R did not show a specific state of hypercoagulation [7.1 min (IQR 5.2 to 8.1) and 6.6 min (IQR 5.2-7.5)] whereas the distribution in percentiles towards a hypercoagulation pattern was evident by examining the FF-MA [42.2 mm (IQR 30.9 to 49.2)], rTEG-Ang [80.5° (IQR 78.7 to 81.5)], and TEG-ACT [78.5 sec (IQR 69.2 to 87.9)] (Table I). Absent lysis of the clot at 30 minutes (LY30) was observed in all the studied population, independently from the outcome (Table I and Table II). By comparing the distribution in percentiles with the Kernel Density Estimate, the value peaks of all the measured parameters showed a hypercoagulation pattern (Figure 1). The angle and maximal clot strength amplitude of the different types of TEG®6s tests have at least 50% of the values distributed above the standard limit, and the distribution of TEG-ACT values show more than 75% of the values below the average values. Altogether, these data were suggestive of a prothrombotic pattern (Table II).

Seven-Day Follow-Up

White blood cell (WBC) count (7.79x10^9·l^{-1} (IQR 6 to 12.25) *vs.* 11.36x10^9·l^{-1} (IQR 9.86-15.89), $p < 0.01$), platelet count (IQR 177.5x10^9·l^{-1} (155 to 250) *vs.* 260.5x10^9·l^{-1} (IQR 214 to 413), $p < 0.001$), neutrophil count (IQR 6.56x10^9·l^{-1} (3.73 to 10.72) *vs.* 8.83x10^9·l^{-1} (IQR 8.11 to 11.73), $p = 0.02$), lymphocyte count (IQR 0.73x10^9·l^{-1} (0.47 to 1.09) *vs.* 1.16x10^9·l^{-1} (IQR 0.62 to 1.54), $p < 0.01$), and D-dimer levels (IQR 1034.5 ng·ml^{-1} (628 to 3762) *vs.* 5330 ng·ml^{-1} (IQR 2187 to 15800), $p = 0.02$) were significantly increased after seven days in the 26 patients with the seven-day follow-up blood samples available. We also noticed a decrease in lactate dehydrogenase (LDH) (IQR 451.5 U·l^{-1} (361 to 554) *vs.* 364.5 U·l^{-1} (IQR 298 to 425), $p = 0.01$), and aPTT (34.9 sec (IQR 29.2 to 42.5) *vs.* 31.4 sec (IQR 28.1 to 36), $p < 0.05$). No infections were described for these patients during the first seven days. These data were collected in 26 patients (Table III).

TEG®6s parameters and variables do not significantly differ at seven-day follow-up, and after a week of full-dose systemic anticoagulation, confirming a state of hypercoagulability explained by the distribution above the normal range of angle and maximal clot strength amplitude (MA). Anticoagulation, either enoxaparin or unfractionated heparin, show no impact on TEG patterns at seven days (Figure 2, Table III).

Table I. Clinical and laboratory characteristics of the study population.

	Total (n=40)	Alive (n= 23)	Dead (n=17)	p
Sex, female. No. (%)	11 (27.5)	6 (26.1)	5 (29.4)	>0.99
Age [IQR]	67.5 [55 to 77]	57 [48 to 67]	77 [75 to 80]	**<0.01***
Cardiovascular disease. No. (%)	21 (52.5)	7 (30.4)	14 (82.4)	**<0.01***
Respiratory disease. No. (%)	6 (15)	0	6 (35.3)	**<0.01***
Obesity. No. (%)	3 (7.5)	2 (8.7)	1 (5.9)	>0.99
Renal disease. No. (%)	2 (5)	0	2 (11.8)	0.18
Diabetes. No. (%)	3 (7.5)	2 (8.7)	1 (5.9)	>0.99
FiO_2. Ratio [IQR]	0.55 [0.5 to 0.6]	0.6 [0.5 to 0.6]	0.5 [0.5 to 0.6]	0.97
OTI. No. (%)	29 (72.5)	15 (65.2)	14 (82.4)	0.23
S_pO_2. % [IQR]	98 [96 to 98.5]	98 [97 to 99]	96 [95 to 98]	**<0.01***
P_aO_2/F_iO_2. Ratio [IQR]	190 [149.5 to 221.5]	196 [178 to 232]	152 [134 to 193]	**0.048***
S_pO_2/F_iO_2. Ratio [IQR]	174 [161.5 to 197]	165 [163 to 198]	188 [160 to 196]	0.64
P_aCO_2. mmHg [IQR] (reference range = 35-48)	36 [32 to 41]	35 [32 to 44]	36 [32 to 39.7]	0.90
Lac. mmol l^{-1} [IQR] (reference range = 0.5-1.6)	1.1 [1 to 1.6]	1.1 [1 to 1.4]	1.1 [0.9 to 1.8]	0.97
pH [IQR] (reference range = 7.35-7.45)	7.42 [7.36 to 7.46]	7.42 [7.38 to 7.44]	7.43 [7.34 to 7.47]	0.65
HR, bpm [IQR]	79 [66 to 86.5]	79 [66 to 87]	79 [69 to 86]	0.52
MAP, mmHg [IQR]	79.5 [77.5 to 86]	82 [78 to 89]	79 [77 to 81]	0.08
Darunavir/Ritonavir. No. (%)	7 (17.5)	6 (26.1)	1 (5.9)	0.21
Lopinavir/Ritonavir. No. (%)	30 (75)	16 (69.6)	14 (82.4)	0.47
Antibiotics. No. (%)	36 (90)	21 (91.3)	15 (88.2)	>0.99
Sarilumab. No. (%)	4 (10)	4 (17.4)	0	0.12
Tocilizumab. No. (%)	7 (17.5)	7 (30.4)	0	**0.01***
DIC score. Mean ± SD	2.9 ± 0.6	2.7 ± 0.7	3.2 ± 0.4	**<0.01***
SOFA score. Mean ± SD	5 ± 2.9	4 ± 2.7	7 ± 2.4	**<0.01***
WBC count. X 10^9 l^{-1} [IQR] (reference range = 4-10)	8.54 [5.98 to 12.68]	7.54 [4.9 to 10.6]	11.08 [6.43 to 15.26]	0.12
Neutrophil count. X 10^9 l^{-1} [IQR] (reference range = 1.5-7)	7.39 [3.99 to 10.91]	6.2 [3.73 to 9.09]	9.75 [5.7 to 12.12]	0.10
Lymphocyte count. X 10^9 l^{-1} [IQR] (reference range = 1.5-3)	0.77 [0.49 to 1.14]	0.85 [0.61 to 1.27]	0.6 [0.38 to 0.84]	0.08
CRP. mg l^{-1} [IQR] (reference range < 5)	160.1 [74.55 to 193.15]	94.8 [41.9 to 185.6]	182 [152.5 to 212]	**0.02***
PCT. ng ml^{-1} [IQR] (reference range < 0.5)	0.23 [0.11 to 0.57]	0.17 [0.09 to 0.8]	0.23 [0.19 to 0.29]	0.30
LDH. U l^{-1} [IQR] (reference range < 250)	463 [344.5 to 566]	441 [317 to 530]	547 [407 to 656]	0.12
aPTT. sec [IQR] (reference range = 20-38)	34.5 [29.7 to 42.2]	33.7 [28.7 to 44.8]	35.4 [31.3 to 41.9]	0.46
INR. Ratio [IQR] (reference range = 0.8-1.2)	1.09 [1.01 to 1.20]	1.07 [1 to 1.15]	1.11 [1.03 to 1.21]	0.29
Fib. mg dl-1 [IQR] (reference range = 200-400)	512.5 [303.5 to 605]	487 [385 to 596]	557 [428 to 756]	0.37
Platelet count. x 10^9 l^{-1} [IQR] (reference range = 150-450)	193.5 [163 to 281]	228 [165 to 347]	185 [161 to 250]	0.34
AT. % [IQR] (reference range = 70-140)	97.5 [89.5 to 115]	100 [94 to 120]	90 [75 to 104]	**0.02***

Table continued

Table I. (Continued). Clinical and laboratory characteristics of the study population.

	Total (n=40)	Alive (n= 23)	Dead (n=17)	p
D-dim. ng ml^{-1} [IQR] (reference range = < 500)	1752.5 [698.5 to 4434.5]	851 [530 to 2714]	3762 [1464 to 6045]	**<0.01***
CK-R time. min [IQR] (reference range = 4.6-9.1)	7.1 [5.2 to 8.1]	6.9 [5.2 to 7.8]	7.8 [5 to 8.3]	0.40
CK-K time. min [IQR] (reference range = 0.8-2.1)	1.1 [0.9 to 1.5]	1.3 [0.9 to 1.5]	1.1 [1 to 1.2]	0.66
CK-Ang. degrees [IQR] (reference range = 63-78)	74.9 [70.9 to 77.5]	74.1 [70.2 to 78.2]	75.2 [73.5 to 77.1]	0.75
LY30. % [IQR] (reference range = 0-2.6)	0 [0-0]	0 [0-0]	0 [0-0]	0.18
rTEG-R. min [IQR] (reference range = 0.3-1.1)	0.3 [0.2 to 0.4]	0.4 [0.2 to 0.4]	0.3 [0.2 to 0.4]	0.20
rTEG-K. min [IQR] (reference range = 0.8-2.7)	0.7 [0.7 to 0.8]	0.8 [0.7 to 0.8]	0.7 [0.6 to 0.8]	0.38
rTEG-Ang. Degrees [IQR] (reference range = 60-78)	80.5 [78.7 to 81.5]	80 [78.3 to 81.3]	81.1 [79.6 to 81.9]	0.20
rTEG-MA. mm [IQR] (reference range = 52-70)	69.8 [66.3 to 71.3]	68.8 [66.2 to 71.8]	70.3 [68.9 to 71]	0.97
TEG-ACT time. sec [IQR] (reference range = 82-152)	78.5 [69.2 to 87.9]	87.9 [69.2 to 87.9]	78.5 [69.2 to 87.9]	0.20
CRT-A10. mm [IQR] (reference range = 44-67)	68.5 [63 to 70.6]	67.3 [62.7 to 71]	69.1 [67.4 to 70]	0.96
CKH-R time. min [IQR] (reference range = 4.3-8.3)	6.6 [5.2 to 7.5]	6.3 [5.5 to 7.4]	6.6 [4.9 to 7.5]	0.43
CKH-K time. min [IQR] (reference range = 0.8-1.9)	1.1 [0.85 to 1.3]	1.1 [0.9 to 1.3]	1.1 [0.8 to 1.1]	0.57
CKH-Ang. degrees [IQR] (reference range = 64-77)	76.2 [74.6 to 77.7]	76 [73.4 to 77]	76.2 [74.8 to 77.9]	0.57
CKH-MA. mm [IQR] (reference range = 52-69)	68.9 [65.8 to 70.2]	67.9 [64.9 to 70.4]	68.9 [66.2 to 69.7]	0.91
FF-MA. mm [IQR] (reference range = 15-32)	42.2 [30.9 to 49.2]	39.1 [29.9 to 49.2]	43.7 [36.4 to 48.7]	0.44
CFF-A10 mm [IQR] (reference range = 15-30)	38.3 [28.4 to 44.4]	35.7 [27.4 to 44.4]	40.7 [33 to 43.4]	0.42

Data are expressed as median [Interquartile Range, IQR], Frequencies No. (%). DIC and SOFA scores are expressed as mean ± standard deviation (± SD). Abbreviations: OTI, orotracheal intubation; LAC, lactates; HR, heart rate; MAP, mean arterial pressure; DIC, disseminated intravascular coagulation; SOFA, sequential organ failure assessment; WBC, white blood cell; CRP, c-reactive protein; PCT, procalcitonin; LDH, lactate dehydrogenase; FIB, fibrinogen; AT, Antithrombin; D-dim, D-dimer; R, reaction time; K, coagulation time; ANG, angle; MA, maximum amplitude; CK, citrated recalcified kaolin-activated blood; rTEG, rapid thromboelastography; ACT, activated clotting time; CRT, citrated recalcified kaolin and tissue factor activated blood; A10, amplitude 10 minutes after clotting time; CKH, citrated recalcified kaolin-activated blood treated with heparinase; FF, functional fibrinogen; CFF, citrated functional fibrinogen; LY30, the percentage decrease in amplitude at 30 minutes post-MA.

Discussion

Several reports have been published focusing particularly on the impact of SARS-CoV-2 on coagulation and thromboembolic complications. Most of the published studies consist of local experiences involving small cohorts of patients. Nowadays, there is increasing interest regarding the potential role of viscoelastic testing as an essential diagnostic tool to better understand the pathophysiology of COVID-19. Of thirteen published studies, six[19-24] chose ROTEM technology, other six[12,14,25-27,40] TEG®5000, and only Salem et al[41] chose TEG®6s to perform the viscoelastic as-

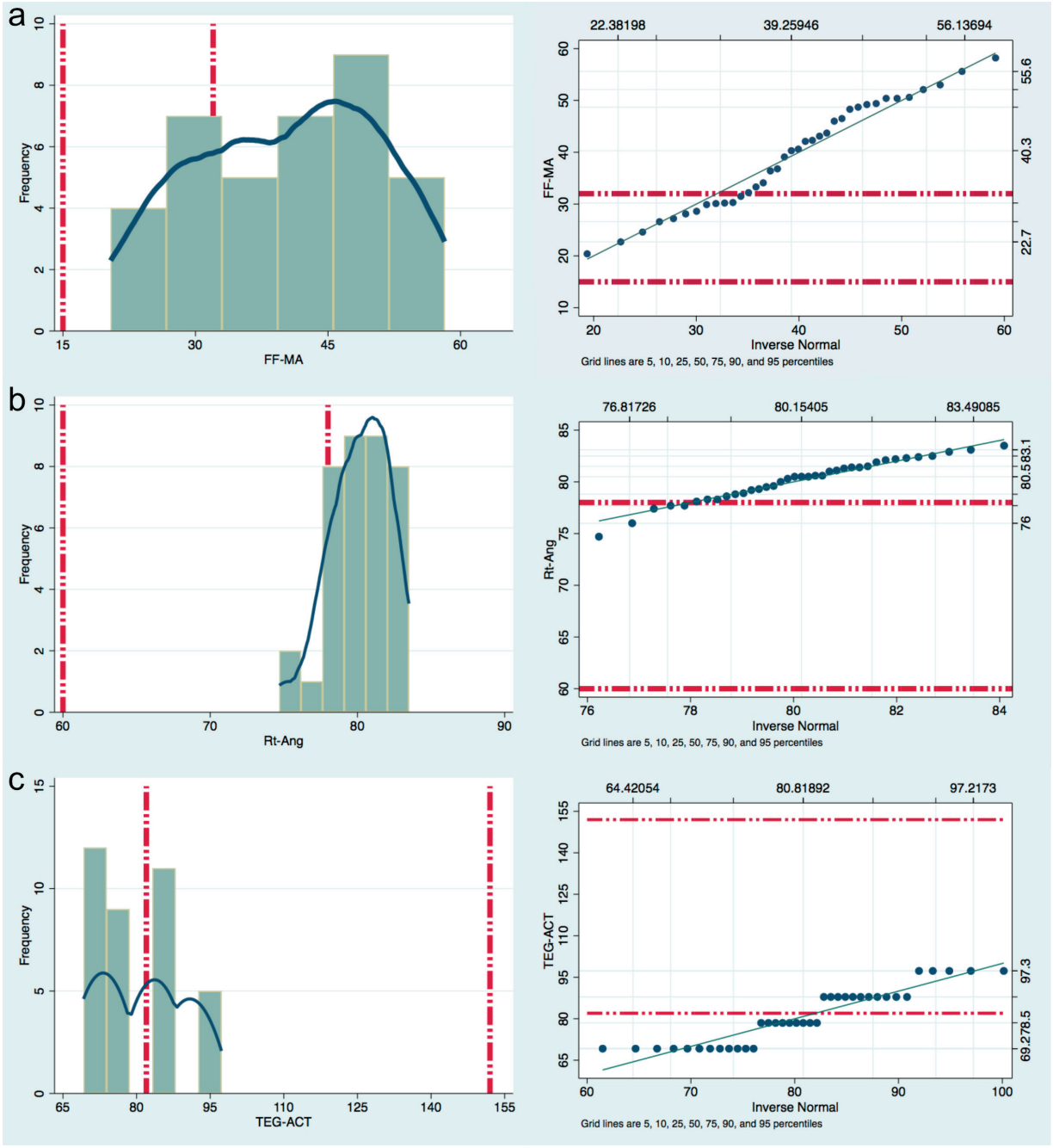

Figure 1. KDE, Frequency and Plot graphs. **a-b:** KDE plus Frequency and Plot graphs showing the distribution of the majority of rTEG-Ang and FF-MA values above the upper limits of the normal range. **c:** Frequency distribution and KDE showing the majority of frequency and density of ACT-TEG values below the lower limit of its normal range. Rt is equivalent to rTEG.

sessment. In this study, we use TEG®6s, a novel and validated device, to perform thromboelastographic analysis in COVID-19 critically ill patients. For the first time, a second assessment at seven-days in 26 out of 40 patients, was performed.

We found that TEG®6s patterns of COVID-19 patients are characterized by an increased amplification phase (MA) in the functional fibrinogen analysis and an increase in angle values in the rTEG. Activated clotting time (TEG-ACT) in rTEG was also reduced[41]. Elevated maximal clot

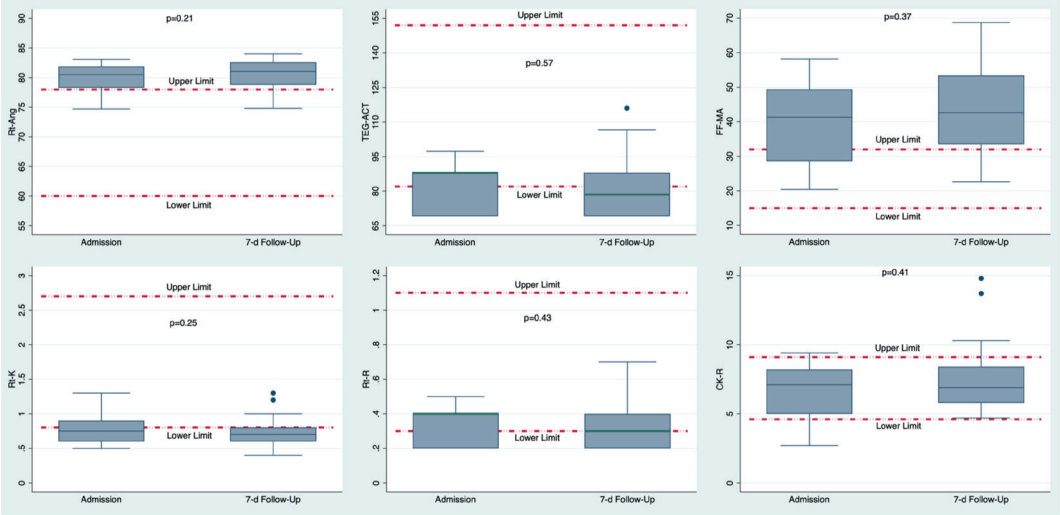

Figure 2. TEG®6s parameters at admission and seven-day follow-up. Box-and-whisker plots at admission to ICU and at seven-day follow up of rTEG-Ang, FF-MA and ACT-TEG in the study population. Dotted lines indicate upper and lower reference ranges. Rt is equivalent to rTEG.

firmness in functional fibrinogen analysis is consistent with the results of previous studies where ROTEM technology was adopted[19-22,27]. Although previous authors[42-46] studying TEG profiles in critically ill patients have proposed elevated maximum amplitude as a marker of hypercoagulability, our results are not strong enough to suggest the presence of an evident prothrombotic state. Brill et al[47] showed that a hypercoagulable thromboelastography, defined as low R, angle or MA above the reference range, was associated with a higher rate of DVT (15.6% *vs.* 8%; $p = 0.039$) in a large study on trauma patients. The combination of the three values was statistically significant[48]. Recently, Panigada et al[26] analyzed CKH patterns in a series of 24 patients and described a global decrease in R and K values. However, consistent with the findings by Salem et al[41] where TEG®6s patterns in a population of 52 Sars-CoV-2 positive patients were analyzed, we did not fully confirm Panigada et al's[26] conclusions regarding CKH-R and CKH-K, as they were in the normal range in our population.

As already discussed by previous studies[20-23], conventional coagulation tests and platelet count were normal in our cohort of patients. Moreover, plasma fibrinogen and D-dimer levels were persistently increased above the reference range, and LY30 remained 0%. Together, higher D-dimer and fibrinogen levels, the absence of clot lysis at 30 minutes, and the observed increase in maximum amplitude, are hallmarks of a state of impaired fibrinolysis. In a recent study on trauma patients, Cotton et al[48] on trauma patients, describe a relation between fibrinolysis shutdown, a profound alteration in fibrinolytic processes, and an increased risk of thrombotic complications. This evidence is also confirmed in the retrospective analysis on 52 patients by Salem et al[41]. In trauma patients, high D-dimer levels and low LY30 already represent criteria to define fibrinolysis shutdown. Wright et al[14] investigated on 44 COVID-19 patients to identify the patients at higher risk of thromboembolic complications. They correlated the thrombotic events with a complete fibrinolysis shutdown, defined by LY30 of 0% and D-dimer levels > 2600 ng·ml^{-1}. In our study, the population with the worst outcome at 28 days presented a complete fibrinolysis shutdown, according to and in agreement of the criteria suggested by Wright et al[14], with an LY30 of 0% and a median D-dimer level of 3762 ng·ml^{-1} (IQR 1464 to 6045).

Different hypotheses have been made to explain high D-dimer levels in SARS-CoV-2 infection. Some authors[49] suggest that, due to the long half-life of D-dimer, they do not reflect the current fibrinolytic activity. Gall et al[50] hypothesized that low fibrinolysis with high D-dimer levels reflects a fibrinolytic process that is not measurable by viscoelastic methods, what they called an "occult hyperfibrinolysis". Ibañez et al[19] proposed a third hypothesis: intra-alveolar fibrin deposition is a common finding in COVID-19 pneumonia and, due to the alveolar damage, alveolar epithelial

cells could be induced to produce urokinase. This determines that the lungs could be the potential source of D-dimer, coexisting with the systemic hypofibrinolytic state.

Although D-dimer was high, contrary to the study by Tang et al[51] and consistently with other experiences, DIC score per ISTH criteria remain lower than 4, with no evidence for consumption coagulopathy[12,14,19,23-25].

Our results support the hypothesis presented by Yuriditsky et al[25] that fibrinogen and platelet have a massive impact on the thromboelastography profiles in COVID-19 syndrome. It is crucial to better understand if conventional anticoagulation can be useful to prevent thromboembolic events. All the patients included in this study received full-dose systemic anticoagulant from ICU admission (Enoxaparin 0.5 $mg \cdot kg^{-1}$ subcutaneous twice daily, unfractionated Heparin 7500 units subcutaneously three times daily, or low-intensity heparin infusion). No changes were found in conventional coagulation teats or TEG variables, after seven days of anticoagulant therapy.

Nougier et al[21] investigated fibrinolytic activity and thrombin generation in 48 COVID-19 critically ill patients. In their brief report, they hypothesized that the coagulopathy could be due to a type of major inflammatory syndrome, in light of high levels of fibrinogen and Factor VIII. At seven-day follow-up, we found a significant increase in white blood cell count, lymphocyte count and platelet count (Table III). Immunomodulatory effect of anticoagulants has been discussed in the literature, and we cannot exclude that the increase in circulating blood cells could be due to UFH or LMWH[52].

COVID-19 viscoelastic profiles appear comparable to those obtained from patients affected by the chronic inflammatory disease, and this is also supported by the evidence of an increase in markers of systemic inflammation, such as platelet count and D-dimer levels at seven-day follow-up. To detect this, Turk et al[53] investigated the presence of a prothrombotic state in patients affected by chronic inflammatory syndromes and also described a higher incidence of thrombotic arterial and venous events.

Klok et al[11] and Shah et al[12] reported, in COVID-19 patients, a rate of thrombotic events of 31% and 43.3%, respectively. We observed only

Table II. Percentages of observed values above upper limits or below lower limits.

	Limits of normal range		
	> Upper limit (%)	< Lower limit (%)	Skewness
CK-R	5	10	-0.39
CK-K	0	10	0.59
CK-Ang	10	0	-0.36
CK-MA	25	0	-0.75
LY30	0	0	3.75
rTEG-R	0	50	0.2
rTEG-K	0	75	1.64
rTEG-Ang	75	0	-0.56
rTEG-MA	50	0	-0.42
TEG-ACT	0	75	0.21
CRT-A10	50	0	-0.57
CKH-R	10	10	-0.1
CKH-K	5	10	1.13
CKH-Ang	25	0	-0.56
CKH-MA	50	0	-0.37
FF-MA	75	0	-0.13
CFF-A10	75	0	-0.08

Negative skewness indicates a left shift of data distribution above upper limits of normal range; positive skewness indicates a right shift of values' distribution below lower limits of normal range. Abbreviations: R, reaction time; K, coagulation time; ANG, angle; MA, maximum amplitude; CK, citrated recalcified kaolin-activated blood; rTEG, rapid thromboelastography; ACT, activated clotting time; CRT, citrated recalcified kaolin and tissue factor activated blood; A10, amplitude 10 minutes after clotting time; CKH, citrated recalcified kaolin-activated blood treated with heparinase; FF, functional fibrinogen; CFF, citrated functional fibrinogen; LY30, the percentage decrease in amplitude at 30 minutes post-MA.

Table III. Laboratory characteristics of the study population assessed at admission and seven-day follow-up (n=26).

	Admission	7-day follow-up	*p*
WBC count. x 10^9 l^{-1} [IQR] (reference range = 4-10)	7.79 [6 to 12.25]	11.36 [9.86 to 15.89]	**<0.01***
Neutrophil count. x 10^9 l^{-1} [IQR] (reference range = 1.5-7)	6.56 [3.73 to 10.72]	8.83 [8.11 to 11.73]	**0.02***
Lymphocyte count. x 10^9 l^{-1} [IQR] (reference range = 1.5-3)	0.73 [0.47 to 1.09]	1.16 [0.62 to 1.54]	**<0.01***
CRP. mg l^{-1} [IQR] (reference range < 5)	156.25 [85 to 193.5]	125.8 [15 to 247]	0.56
PCT. ng ml^{-1} [IQR] (reference range < 0.5)	0.25 [0.17 to 0.87]	0.28 [0.17 to 0.49]	0.38
LDH. U l^{-1} [IQR] (reference range < 250)	451.5 [361 to 554]	364.5 [298 to 425]	**0.01***
aPTT. sec [IQR] (reference range = 20-38)	34.9 [29.2 to 42.5]	31.4 [28.1 to 36]	**0.03***
INR. Ratio [IQR] (reference range = 0.8-1.2)	1.08 [1 to 1.21]	1.07 [1.02 to 1.14]	0.61
Fib. mg dl^{-1} [IQR] (reference range = 200-400)	554 [385 to 624]	571 [322 to 696]	0.87
Platelet count. x 10^9 l^{-1} [IQR] (reference range = 150-450)	177.5 [155 to 250]	260.5 [214 to 413]	**<0.01***
AT. % [IQR] (reference range = 70-140)	98.5 [90 to 114]	101.5 [93 to 108]	0.77
D-dim. ng ml^{-1} [IQR] (reference range = < 500)	1034.5 [628 to 3762]	5330 [2187 to 15800]	**0.02***
CK-R time. min [IQR] (reference range = 4.6-9.1)	7.1 [5 to 8.2]	6.9 [5.8 to 8.4]	0.19
CK-K time. min [IQR] (reference range = 0.8-2.1)	1.15 [1.1 to 1.5]	0.95 [0.8 to 1.5]	0.50
CK-Ang. degrees [IQR] (reference range = 63-78)	74.7 [70.2 to 76.2]	75.05 [71.1 to 78]	0.67
CK-MA. mm [IQR] (reference range = 52-69)	68.25 [64.4 to 69.7]	69.5 [66.3 to 72.6]	0.09
rTEG-R. min [IQR] (reference range = 0.3-1.1)	0.4 [0.2 to 0.4]	0.3 [0.2 to 0.4]	0.49
rTEG-K. min [IQR] (reference range = 0.8-2.7)	0.75 [0.6 to 0.9]	0.7 [0.6 to 0.8]	0.38
rTEG-Ang. Degrees [IQR] (reference range = 60-78)	80.5 [78.3 to 81.9]	81.05 [78.8 to 82.6]	0.13
rTEG-MA. mm [IQR] (reference range =52-70)	69.75 [65.7 to 71.2]	70.35 [68.1 to 73.4]	0.10
TEG-ACT time. sec [IQR] (reference range = 82-52)	87.9 [69.2 to 87.9]	78.5 [69.2 to 87.9]	0.58
CRT-A10. mm [IQR] (reference range = 44-67)	68.45 [61.7 to 70.6]	69.5 [64.3 to 72.9]	0.21
CKH-R time. min [IQR] (reference range = 4.3-8.3)	6.6 [4.9 to 7.5]	6.2 [5.7 to 8.1]	0.35
CKH-K time. min [IQR] (reference range = 0.8-1.9)	1.1 [0.9 to 1.3]	1.05 [0.9 to 1.3]	0.82

Table continued

Table III. ***(Conintued).*** Laboratory characteristics of the study population assessed at admission and seven-day follow-up (n=26).

	Admission	7-day follow-up	p
CKH-Ang. degrees [IQR] (reference range = 64-77)	75.79 [74.3 to 76.7]	77.05 [73 to 79.4]	0.75
CKH-MA. mm [IQR] (reference range = 52-69)	68.45 [64.6 to 70.1]	69.2 [65.9 to 71.9]	0.24
FF-MA. mm [IQR] (reference range = 15-32)	41.3 [28.6 to 49.4]	42.6 [33.5 to 53.4]	0.24
CFF-A10 mm [IQR] (reference range = 15-30)	37.8 [25.8 to 45]	38.6 [31.5 to 51.4]	0.24

Data are expressed as median [Interquartile Range, IQR]. Abbreviations: WBC, white blood cell; CRP, c-reactive protein; PCT, procalcitonin; LDH, lactate dehydrogenase; FIB, fibrinogen; AT, Antithrombin; D-dim, D-dimer; R, reaction time; K, coagulation time; ANG, angle; MA, maximum amplitude; LY30, percentage of lysis 30 minutes after MA was finalized; CK, citrated recalcified kaolin-activated blood; RT, rapid thromboelastography; ACT, activated clotting time; CRT, citrated recalcified kaolin and tissue factor activated blood; A10, amplitude 10 minutes after clotting time; CKH, citrated recalcified kaolin-activated blood treated with heparinase; FF, functional fibrinogen; CFF, citrated functional fibrinogen.

two cases of pulmonary embolism, and no evidence of deep venous thrombosis was found.

However, some limitations of this study should be noted. First, this is a small single-center study. Despite the fact that we enrolled consecutive patients trying to avoid possible selection bias, the number remains limited. We observed a lower incidence of thromboembolic events, and this can be due mainly to the fact that we do not perform routinely computed tomography pulmonary angiogram or ultrasound assessments. On the other hand, our cohort of patients was limited compared to the population included in the study by Klok et al[11] and Shah et al[12]. We could not compare the results with a control group, and only in 26 patients the follow-up analysis was available. TEG®6s allows easily to perform more tests with a single blood sample and a single cartridge, eliminating the bias of the pre-analytic phase but has got limits. As seen in the study published by Lloyd-Donald et al[32] TEG®6s and TEG®5000 are interchangeable, except for some apparent bias in MA (difference 5.2 mm) and difference in LY30 (0.61%).

Conclusions

TEG®6s, a novel device, allowed us to study the fibrinogen contribution to clot formation through TEG functional fibrinogen, an essential tool that is not available using TEG®5000. For the first time we assessed the impact of a full-dose systemic anticoagulation on viscoelastic parameters. An integrated approach allows the identification of fibrinolysis shutdown in the early phases of the infectious disease. This pattern is not characterized by platelet, fibrinogen, and coagulation factors consumption that are typical of DIC. On the contrary, hypofibrinolysis together with increased D-dimer values are typical hallmarks of this disease. Whether these abnormalities are predictive for thrombotic events as part of an inflammatory process is an interesting hypothesis which needs further ad hoc designed studies. Chow et al[54], in a recent large retrospective study, suggested that aspirin use may be associated with improved outcomes in hospitalized COVID-19 patients. Unfortunately, we have no data on the effects of routinely given antiplatelets medications in COVID-19 syndrome in our center. As a consequence, there is a great need to investigate the effect of antiplatelets and other antithrombotic medications, as low molecular weight heparin and unfractionated heparin show no effects.

Ethics Approval and Consent to Participate

The study protocol was approved by the local review board (Ethics Committee at Fondazione Policlinico Universitario A. Gemelli IRCCS, protocol id number 3146), and informed consent was obtained for each individual enrolled in the study.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contributions

MGB, LMC, CG, and LP designed the study, developed the protocol and drafted the first version of the manuscript; LM, SLC and GM provided professional input on the protocol development and methodology; RM, DLG, DGB performed the statistical analysis; ED and SC provided professional expertise in the analysis of TEG®6s results; DN, RR, GN, FF and MA provided professional expertise in the writing process of the paper and in the critical analysis of the results. All authors comply with the ICMJE recommendations: a) all authors provided a substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data; b) all authors drafted the article or revised it critically for important intellectual content; c) all authors have given final approval of the version to be published, and d) all authors agreed to be accountable for all aspects of the work thereby ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved..

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References

1) Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: their roles in pathogenesis. J Microbiol Immunol Infect 2020 Mar 31. doi: 10.1016/j.jmii.2020.03.022. Online ahead of print.
2) Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. A novel Coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020; 382: 727-733.
3) Tomashefski JF Jr, Davies P, Boggis C, Greene R, Zapol WM, Reid LM. The pulmonary vascular lesions of the adult respiratory distress syndrome. Am J Pathol 1983; 112: 112-126.
4) Fung SY, Yuen KS, Ye ZW, Chan CP, Jin DY. A tug-of-war between severe acute respiratory syndrome coronavirus 2 and host antiviral defence: lessons from other pathogenic viruses. Emerg Microbes Infect 2020; 9: 558-570.
5) Robriquet L, Guery B, Fourrier F. Pulmonary coagulopathy as a new target in therapeutic studies of acute lung injury or pneumonia. Crit Care Med 2006; 34: 2510-2511.
6) Yin S, Huang M, Li D, Tang N. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. J Thromb Thrombolysis 2020; 1-4. doi: 10.1007/s11239-020-02105-8. Online ahead of print.
7) Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013; 369: 840-851.
8) Bakhtiari K, Meijers JC, de Jonge E, Levi M. Prospective validation of the International Society of Thrombosis and Haemostasis scoring system for disseminated intravascular coagulation. Crit Care Med 2004; 32: 2416-2421.
9) Ostrowski SR, Windeløv NA, Ibsen M, Haase N, Perner A, Johansson PI. Consecutive thrombelastography clot strength profiles in patients with severe sepsis and their association with 28-day mortality: a prospective study. J Crit Care 2013; 28: 317.e1-11. doi: 10.1016/j.jcrc.2012.09.003.
10) Grasselli G, Tonetti T, Protti A, Langer T, Girardis M, Bellani G, Laffey J, Carrafiello G, Carsana L, Rizzuto C, Zanella A, Scaravilli V, Pizzilli G, Grieco DL, Di Meglio L, de Pascale G, Lanza E, Monteduro F, Zompatori M, Filippini C, Locatelli F, Cecconi M, Fumagalli R, Nava S, Vincent JL, Antonelli M, Slutsky AS, Pesenti A, Ranieri VM; collaborators. Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. Lancet Respir Med 2020; S2213-2600(20)30370-2.
11) Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, Kaptein FHJ, van Paassen J, Stals MAM, Huisman MV, Endeman H. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020; 91: 145-147.
12) Shah A, Donovan K, McHugh A, Pandey M, Aaron L, Bradbury CA, Stanworth SJ, Alikhan R, Von Kier S, Maher K, Curry N, Shapiro S, Rowland MJ, Thomas M, Mason R, Holland M, Holmes T, Ware M, Gurney S, McKechnie SR. Thrombotic and haemorrhagic complications in critically ill patients with COVID-19: a multicentre observational study. Crit Care 2020; 24: 561.
13) Maatman TK, Jalali F, Feizpour C, Douglas A 2nd, McGuire SP, Kinnaman G, Hartwell JL, Maatman BT, Kreutz RP, Kapoor R, Rahman O, Zyromski NJ, Meagher AD. Routine venous thromboembolism prophylaxis may be inadequate in the hypercoagulable state of severe Coronavirus disease 2019. Crit Care Med 2020; 48: e783-e790. doi: 10.1097/CCM.0000000000004466.
14) Wright FL, Vogler TO, Moore EE, Moore HB, Wohlauer MV, Urban S, Nydam 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.
15) Johansson PI, Stensballe J, Vindeløv N, Perner A, Espersen K. Hypocoagulability as evaluated by thrombelastography, at admission to the ICU is associated with increased 30-day mortality. Blood Coagul Fibrinolysis 2010; 21: 168-174.
16) Müller MC, Meijers, JCM, Vroom MB, Juffermans NP. Utility of thromboelastography and/or thromboelastometry in adults with sepsis: a systematic review. Crit Care 2014; 18: R30.
17) Haase N, Ostrowski SR, Wetterslev J, Lange T, Møller MH, Tousi H, Steensen M, Pott F, Søe-Jensen P, Nielsen J,

Hjortrup PB, Johansson PI, Perner A. Thromboelastography in patients with severe sepsis: a prospective cohort study. Intensive Care Med 2014; 41: 77-85.

18) Cecconi M, Evans L, Levy M, Rhodes A. Sepsis and septic shock. Lancet 2018; 392: 75-87.

19) Ibañez C, Perdomo J, Calvo A, Ferrando C, Reverter JC, Tassies D, Blasi A. High D dimers and low global fibrinolysis coexist in COVID19 patients: what is going on in there? J Thromb Thrombolysis 2020; 1-5. doi: 10.1007/s11239-020-02226-0. Online ahead of print.

20) Collett LW, Gluck S, Strickland RM, Reddi BJ. Evaluation of coagulation status using viscoelastic testing in intensive care patients with coronavirus disease 2019 (COVID-19): an observational point prevalence cohort study. Aust Crit Care 2020; S1036-7314(20)30254-X. doi: 10.1016/j.aucc.2020.07.003. Online ahead of print.

21) Nougier C, Benoit R, Simon M, Desmurs-Clavel H, Marcotte G, Argaud L, David JS, Bonnet A, Negrier C, Dargaud Y. Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. J Thromb Haemost 2020; 10.1111/jth.15016. doi: 10.1111/jth.15016. Online ahead of print.

22) Corrêa TD, Cordioli RL, Campos Guerra JC, Caldin da Silva B, dos Reis Rodrigues R, de Souza GM, Midega T, Campos N, Carneiro B, Campos F, Guimaraes H, Matos G, Aranda V, Ferraz L. The hypercoagulability state of COVID-19 ICU patients is characterized by impaired endogenous anticoagulation and decreased fibrinolysis. Research Square 2020; doi: 10.21203/rs.3.rs-47465/v1.

23) Pavoni V, Gianesello L, Pazzi M, Stera C, Meconi T, Frigieri FC. Evaluation of coagulation function by rotation thromboelastometry in critically ill patients with severe COVID-19 pneumonia. J Thromb Thrombolysis 2020; 50: 281-286.

24) Spiezia L, Boscolo A, Poletto 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.

25) Yuriditsky E, Horowitz JM, Merchan C, Ahuja T, Brosnahan SB, McVoy L, Berger JS. Thromboelastography profiles of critically ill patients with Coronavirus disease 2019. Crit Care Med 2020; 48: 1319-1326.

26) 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; 1738-1742.

27) Mortus JR, Manek SE, Brubaker LS, Loor M, Cruz MA, Trautner BW, Rosengart TK. Thromboelastographic results and hypercoagulability syndrome in patients with Coronavirus disease 2019 who are critically ill. JAMA Netw Open 2020; 3: e2011192.

28) Quarterman Q, Shaw M, Johnson I, Agarwal S. Intra- and inter-centre standardization of thromboelastography (TEG®). Anaesthesia 2014; 69: 883-890.

29) Blasio E, Pellegrini C, Federico A, Rocco V, Fumi M, Pancione Y, Sale S, Liberti D. Coagulation support algorithm with rapid TEG and functional fibrinogen TEG in critical bleeding: more results and less time. Crit Care 2015; 19: P352.

30) Hans GA, Besser MW. The place of viscoelastic testing in clinical practice. Br J Haematol 2016; 173: 37-48.

31) Gurbel PA, Bliden KP, Tantry US, Monroe AL, Muresan AA, Brunner NE, Lopez-Espina CG, Delmenico PR, Cohen E, Raviv G, Haugen DL, Ereth MH. First report of the point-of-care TEG: A technical validation study of the TEG-6S system. Platelets 2016; 27: 642-649.

32) Lloyd-Donald P, Churilov L, Zia F, Bellomo R, Hart G, McCall P, Mårtensson J, Glassford N, Weinberg L. Assessment of agreement and interchangeability between the TEG5000 and TEG6S thromboelastography haemostasis analyzers: a prospective validation study. BMC Anesthesiol 2019; 19: 45.

33) ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012; 307: 2526-2533.

34) Taylor FB, Toh CH, Hoots WK, Wada H, Levi M. Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost 2001; 86: 1327-1330.

35) Toh CH, Hoots WK. SSC on Disseminated Intravascular Coagulation of the ISTH. The scoring system of the Scientific and Standardisation Committee on Disseminated Intravascular Coagulation of the International Society on Thrombosis and Haemostasis: a 5-year overview. J Thromb Haemost 2007; 5: 604-606.

36) Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. Intensive Care Med 1996; 22: 707-710.

37) Barnes GD, Burnett A, Allen A, Blumenstein M, Clark NP, Cuker A, Dager WE, Deitelzweig SB, Ellsworth S, Garcia D, Kaatz S, Minichiello T. Thromboembolism and anticoagulant therapy during the COVID-19 pandemic: interim clinical guidance from the anticoagulation forum. J Thromb Thrombol 2020; 50: 72-81.

37) Chaudhary R, Kreutz RP, Bliden KP, Tantry US, Gurbel PA. Personalizing antithrombotic therapy in COVID-19: role of thromboelastography and thromboelastometry. Thromb Haemost 2020 Jul 17; doi: 10.1055/s-0040-1714217. Online ahead of print.

39) Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharmaceutical Statistics. 2005; 4: 287-291.

40) Sadd C, Rowe T, Nazeef M, Kory P, Sultan S, Faust H. Thromboelastography to detect hypercoagula-

bility and reduced fibrinolysis in Coronavirus disease 2019 acute respiratory distress syndrome patients. Crit Care Explor 2020; 2: e0192.

41) Salem N, Atallah B, El Nekidy WS, Sadik ZG, Park WM, Mallat J. Thromboelastography findings in critically ill COVID-19 patients. J Thromb Thrombolysis 2020: 1-5. doi:10.1007/s11239-020-02300-7. Online ahead of print.

42) Ostrowski SR, Windelov NA, Ibsen M, Haase N, Perner A, Johansson PI. Consecutive thromboelastographycclot strength profiles in patients with severe sepsis and their association with 28-day mortality: a prospective study. J Crit Care 2013; 28: 317.e1-11.

43) Halset JH, Hanssen SW, Espinosa A, Klepstad P. Thromboelastography. Variability and relation to conventional coagulation tests in non-bleeding intensive care unit patients. BMC Anesthesiol 2015; 15: 28.

44) Crochemore T, Corrêa TD, Lance MD, Solomon C, Neto AS, de Campos Guerra JC, Lellis PS, Bernz LM, Nunes N, Mancio CM, Yokoyama APH, Silva E. Thromboelastometry profile in critically ill patients: A single-center, retrospective, observational study. PLoS One 2018; 13: e0192965.

45) McCrath DJ, Cerboni E, Frumento RJ, Hirsh AL, Bennett-Guerrero E. Thromboelastography maximum amplitude predicts postoperative thrombotic complications including myocardial infarction. Anesth Analg 2005; 100: 1576e1583.

46) Harahsheh Y, Duff OC, Ho KM. Thromboelastography predicts thromboembolism in critically ill coagulopathic patients. Crit Care Med 2019; 47: 826-832.

47) Brill JB, Badiee J, Zander AL, Wallace JD, Lewis PR, Sise MJ, Bansal V, Shackford SR. The rate of deep vein thrombosis doubles in trauma patients with hypercoagulable thromboelastography. J Trauma Acute Care Surg 2017; 83: 413-419.

48) Cotton BA, Minei KM, Radwan ZA, Matijevic N, Pivalizza E, Podbielski J, Wade CE, Kozar RA, Holcomb JB. Admission rapid thrombelastography predicts development of pulmonary embolism in trauma patients. J Trauma Acute Care Surg 2012; 72: 1470-1475.

49) Rühl H, Berens C, Winterhagen A, Müller J, Oldenburg J, Pötzsch B. Label-free kinetic studies of hemostasis-related biomarkers including D-Dimer using autologous serum transfusion. PLoS One 2015; 10: e0145012.

50) Gall LS, Vulliamy P, Gillespie S, Jones TF, Pierre RSJ, Breukers SE, Gaarder C, Juffermans NP, Maegele M, Stensballe J, Johansson PI, Davenport RA, Brohi K. The S100A10 pathway mediates an occult hyperfibrinolytic subtype in trauma patients. Ann Surg 2019; 269: 1184-1191.

51) 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: 844-847.

52) Li, Xu, Ma, Xiaochun. The role of heparin in sepsis: much more than just an anticoagulant. Br J Haematol 2017; 179: 389-398.

53) Türk SM, Cansu DÜ, Teke HÜ, Kaşifoğlu T, Akay OM, Bilgim M, Korkmaz C. Can we predict thrombotic tendency in rheumatoid arthritis? A thromboelastographic analysis (with ROTEM). Clin Rheumatol 2018; 37: 2341-2349.

54) Chow JH, Khanna AK, Kethireddy S, Yamane D, Levine A, Jackson AM, McCurdy MT, Tabatabai A, Kumar G, Park P, Benjenk I, Menaker J, Ahmed N, Glidewell E, Presutto E, Cain S, Haridasa N, Field W, Fowler JG, Trinh D, Johnson KN, Kaur A, Lee A, Sebastian K, Ulrich A, Peña S, Carpenter R, Sudhakar S, Uppal P, Fedeles BT, Sachs A, Dahbour L, Teeter W, Tanaka K, Galvagno SM, Herr DL, Scalea TM, Mazzeffi MA. Aspirin use is associated with decreased mechanical ventilation, ICU admission, and in-hospital mortality in hospitalized patients with COVID-19. Anesth Analg 2020; doi: 10.1213/ANE.0000000000005292. Online ahead of print.