# Dynamic Hemostasis and Fibrinolysis Assays in Intensive Care COVID-19 Patients and Association with Thrombosis and Bleeding—A Systematic Review and a Cohort Study

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## Abstract

Patients admitted to the intensive care unit (ICU) with coronavirus disease 2019 (COVID-19), the infectious pathology caused by severe acute respiratory syndrome coronavirus 2, have a high risk of thrombosis, though the precise mechanisms behind this remain unclarified. A systematic literature search in PubMed and EMBASE identified 18 prospective studies applying dynamic coagulation assays in ICU COVID-19 patients. Overall, these studies revealed normal or slightly reduced primary hemostasis, prolonged clot initiation, but increased clot firmness. Thrombin generation assay parameters generally were equivalent to the control groups or within reference range. Fibrinolysis assays showed increased clot resistance. Only six studies related their findings to clinical outcome. We also prospectively included 51 COVID-19 patients admitted to the ICU. Blood samples were examined on day 1, 3–4, and 7–8 with platelet function tests, rotational thromboelastometry (ROTEM), in vivo and ex vivo thrombin generation, and clot lysis assay. Data on thrombosis, bleeding, and mortality were recorded during 30 days. Primary hemostasis was comparable to healthy controls, but COVID-19 patients had longer ROTEM-clotting times and higher maximum clot firmness than healthy controls. Ex vivo thrombin generation was similar to that of healthy controls while in vivo thrombin generation markers, thrombin–antithrombin (TAT) complex, and prothrombin fragment $1+2$ ($F1+2$) were higher in ICU COVID-19 patients than in healthy controls. Impaired fibrinolysis was present at all time points. TAT complex and $F1+2$ levels were significantly higher in patients developing thrombosis ($n=16$) than in those without. In conclusion, only few previous studies employed dynamic hemostasis assays in COVID-19 ICU-patients and failed to reveal a clear association with development of thrombosis. In ICU COVID-19 patients, we confirmed normal platelet aggregation, while in vivo thrombin generation was increased and fibrinolysis decreased. Thrombosis may be driven by increased thrombin formation in vivo.

**Keywords**
- COVID-19
- blood coagulation
- thrombin generation
- thrombosis
- intensive care unit



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During the first wave of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19), it became evident that thrombosis is a frequent complication, particularly in patients with critical illness.[1] Changes in coagulation parameters were found to be associated with severity of disease[2,3] and mortality.[4] Early studies reported that patients with COVID-19 have increased fibrin D-dimer and fibrinogen, and a decline in platelet count as a late and poor prognostic sign.[5,6] Hence, the coagulopathy of COVID-19 appears to differ from septic coagulation derangement as it presents as hypercoagulability rather than consumptive coagulopathy.

The dynamic process of coagulation from activation of platelets and clotting factors to fibrin formation, clot stabilization, and fibrinolysis can only be exhaustively examined using a combination of assays, such as platelet aggregometry, global whole blood coagulation assays (e.g., thromboelastography [TEG] or rotational thromboelastometry [ROTEM]), thrombin generation assays, and clot lysis assays. Platelet aggregation has only been sparsely investigated in intensive care unit (ICU) COVID-19 patients and with conflicting results.[7–9] TEG and ROTEM have revealed preserved or slightly prolonged clotting times, but increased clot firmness and indications of hypofibrinolysis. However, the commercially available TEG and ROTEM assays are poor indicators of changes in fibrinolysis.[10,11] Modified TEG/ROTEM assays with addition of tissue plasminogen activator (tPa) have emerged, as well as plasma-based clot lysis assays sensitive to changes in clot formation and lysis, reflecting the global fibrinolytic potential.[11,12] Although several previous studies have examined the hemostasis of COVID-19 patients, many of these were small in size ($n < 20$) and, importantly, lacked data on association between hemostasis parameters and clinical outcomes. Furthermore, none of these previous investigations has explored the whole process of coagulation, from platelet aggregation, coagulation activity, and thrombin generation to fibrinolysis, further linking this to development of thrombosis. Such data are important to elucidate mechanisms behind COVID-19-related coagulopathy and guide strategies for prevention of thrombosis and bleeding.[13,14]

In this study, we examined the hemostasis in ICU COVID-19 patients with a wide range of dynamic hemostasis assays including platelet aggregation, global whole blood coagulation, thrombin generation assays, and fibrinolysis assays. Further, we examined the association between the measured hemostasis parameters and thrombosis or hemorrhage within 30 days. To contextualize our findings to prior studies, we also provide a systematic review of prospective studies on ICU COVID-19 patients employing dynamic assays.

## Methods

### Cohort Study

This prospective cohort study was approved by the Regional Ethics Committee, Central Region Denmark (Project ID 1–10–72–93–20) and the Data Protection Agency. Written informed consent was obtained from the patients or their legal proxies and the study was performed in accordance with the Helsinki Declaration and the Danish Health Care Act. Patients were included from April 8, 2020 to February 15, 2021, during the first and second waves of COVID-19 in Denmark.

### Design and Study Population

Critically ill patients admitted to the ICU at Aarhus University Hospital, Denmark, a tertiary referral hospital, with polymerase chain reaction-confirmed SARS-CoV-2 infection were included if they (1) had moderate or severe acute respiratory distress syndrome due to SARS-CoV-2, and (2) were $\geq$18 years of age. Exclusion criteria were: (1) primary reason for transfer to the ICU was secondary infection, other than SARS-CoV-2, (2) extracorporeal membrane oxygenation (ECMO) treatment had already begun at another ECMO center, (3) prior participation in the present study. Hence, patients were also included if transferred from other ICUs due to capacity problems or evaluation of the need for ECMO.

All patients were treated at the discretion of the ICU team in collaboration with specialists from the Department of Infectious Diseases. From April 15 2020, thromboprophylaxis was increased from dalteparin 5,000 IU/day to 5,000 IU $\times$2/day, and from June 16 2020, dexamethasone 10 mg daily for 10 days and from July 1 2020, remdesivir was added as standard treatment. Antithrombotic therapy was prescribed at the attending physician's discretion.

### Clinical Data

Clinical variables were collected from medical records and ICU observation charts. This included Simplified Acute Physiology Score-III[15] at admission, Sequential Organ Failure Assessment (SOFA) score[16] on each day of blood sampling, thrombotic or bleeding events within 30 days of admission to the ICU, and 30-day mortality. Further, we registered comorbidities according to the updated Charlson comorbidity index,[17] treatment for hypertension, body mass index (BMI), occurrence of thrombosis prior in life or hemorrhage requiring hospitalization within the last year prior to study enrolment, and anticoagulant medications both before and during the hospital stay. During the ICU stay, details on ventilation therapy, including ECMO, blood pressure management, and renal replacement therapy were obtained and the International Society of Thrombosis and Hemostasis disseminated intravascular coagulation (ISTH DIC) score calculated.[18] Occurrence of thrombosis from hospital admission and until 30 days after inclusion was classified as arterial or venous and subclassified according to anatomical location. Verification of the diagnosis by relevant imaging techniques (e.g., ultrasound, computed tomography scan, echocardiography) or maintenance of therapeutic dosing of anticoagulation due to clinical suspicion was required. Imaging was obtained at the attending physician's discretion. Bleeding complications were registered and classified according to the Global Utilization of Streptokinase and tPa for Occluded arteries (GUSTO) bleeding criteria[19] and anatomical location.

### Blood Sampling

Blood samples were obtained on the first morning possibly within 48 hours of admission to the ICU. Blood was drawn from a nonheparinized arterial cannula already in place on the routine morning round at the same time point every day, right before administration of low-molecular-weight heparin (LMWH). A second sample was obtained on the third or fourth morning of admission, and a third sample on the seventh or eighth day. Due to the need for specialized technicians for analysis, patients were included only on weekdays.

### Platelet Aggregation

Impedance aggregometry (Multiplate Analyzer, Roche, Germany): blood was collected in hirudinated tubes (Monovette 1.6 mL, Sarstedt, Nümbrecht, Germany), left to stand for 30 minutes and analyzed within 2 hours, according to manufacturer's instructions. Platelets were activated with adenosine diphosphate (ADP) (Roche ADPtest), collagen (Bio/Data Corporation, BioNordika, Herlev, Denmark), and arachidonic acid (AA) (Roche ASPItest), according to the manufacturer's standard protocols. The area under the curve (AUC; aggregation units*min) was registered.

Light transmission aggregometry (Platelet Aggregation Profiler [PAP8], Bio/Data Corporation, PA): blood was collected in 3.6 mL citrated tubes (sodium citrate 3.2%, Greiner Bio-One, Kremsmünster, Austria) of which one was centrifuged at 150 g for 10 minutes to obtain platelet-rich plasma and one at 2,500 g for 20 minutes to obtain platelet-poor plasma for blank correction. Platelets were activated with ADP (Bio/Data), collagen (Bio/Data), and AA (Bio/Data) according to the manufacturer's standard protocols. Maximal aggregation (%) was registered.

### Rotational Thromboelastometry

Blood for ROTEM (Instrumentation Laboratory, Bedford, MA) was collected in 3.6 mL citrated tubes (Greiner Bio-One, Kremsmünster, Austria), left to rest for 30 minutes, and analyzed within 2 hours. Standard protocols for EXTEM, INTEM, FIBTEM, and HEPTEM were performed according to the manufacturer's instructions. The following parameters were registered: clotting time (seconds), time to maximum velocity (t-MaxVel, seconds), maximum velocity (MaxVel), maximum clot formation (MCF, mm), and maximum lysis (%).

### Thrombin Generation Markers

Blood was collected in citrated tubes (sodium citrate 3.2%, Greiner Bio-One, Kremsmünster, Austria), centrifuged at 3,000 g for 25 minutes within 1 hour to obtain platelet-poor plasma, aliquoted, and stored at −80°C until analysis.

The ex vivo thrombin generation assay (calibrated automated thrombogram, Thrombinoscope BV, Maastricht, the Netherlands) was performed as previously described, with final concentration of 5 pM tissue factor (TF) in wells.[20] Lag time (minutes), time to peak thrombin concentration (minutes), peak thrombin concentration (nM), and endogenous thrombin potential (ETP) (AUC, nM*min) were registered.

In vivo thrombin generation was determined by thrombin–antithrombin (TAT) complex and prothrombin fragment 1 + 2 (F1 + 2) plasma concentrations analyzed with commercial enzyme-linked immunosorbent assay (ELISA) kits (Enzygnost Siemens, Marburg, Germany), according to the manufacturer's instructions.

### Fibrinolysis

Blood was collected in citrated tubes (sodium citrate 3.2%, Greiner Bio-One, Kremsmünster, Austria), centrifuged at 3,000 g for 25 minutes within 1 hour to obtain platelet-poor plasma, aliquoted, and stored at −80°C until analysis. Ex vivo fibrin formation and lysis (clot lysis) were analyzed using our in-house turbidimetric assay.[12] Briefly, final concentrations and dilutions were as follows: TF final dilution 1:5,000 (Siemens, Marburg, Germany), phospholipids, 4 µM (Rossix, Mölndal, Sweden); tPa, 116 ng/mL (Calbiochem, San Diego, CA); $Ca^{2+}$, 26.7 mM. Absorbance was read at 405 nm, 1/min for 90 minutes (Victor microplate reader, Perkin Elmer, Waltham, MA). The analysis was performed in duplicate, and internal controls were added to all plates. The following parameters were derived: peak absorbance corresponding to peak fibrin formation (absorbance units [AU]); AUC corresponding to net fibrin formation (AU*seconds); and time from peak fibrin formation to 50% lysis of the clot (seconds). Lysis-resistant curves were assigned a 50% lysis time of 3,000 seconds, equivalent to the runtime of the analysis.

Plasminogen activator inhibitor (PAI)-1 and plasmin–antiplasmin (PAP) complex levels were analyzed with commercial ELISA kits (Technozyme, Technoclone, Vienna, Austria) according to the manufacturer's instructions.

### Data from Healthy Controls

Impedance aggregometry: data from 50 healthy individuals served as controls.

Light aggregometry: data from 50 healthy blood donors enrolled from the Department of Clinical Immunology, Aarhus University Hospital and used for our in-house reference interval served as controls.

ROTEM: data from 50 healthy individuals served as healthy controls.

Thrombin generation markers: data from 90 (ex vivo thrombin generation assay) and 124 (F1 + 2, TAT complex) healthy individuals previously published by our group[20,21] served as controls.

Fibrinolysis: for the clot-lysis assays, data from 120 healthy individuals previously published by our group[22] served as controls. For PAI-1 and PAP complex, the manufacturer's reference ranges were used.

### Routine Laboratory Analyses

Platelet count, immature platelet count and fraction, mean platelet volume (MPV), international normalized ratio (INR), activated partial thromboplastin time (aPTT), antithrombin, fibrinogen, fibrin D-dimer, anti-factor Xa activity, hemoglobin, and markers of inflammation and organ dysfunction were analyzed at the automated routine laboratory, Department of Clinical Biochemistry, Aarhus University Hospital, according to ISO:15189-accredited protocols.[23] Blood hemoglobin, leukocyte count, and platelet count and indices were

analyzed on Sysmex XN-9000 (Sysmex, Kobe, Japan). INR (Medirox Owren's PT reagent), aPTT (Siemens Dade Actin FS reagent), fibrinogen (functional, Clauss, Siemens Dade thrombin reagent), fibrin D-dimer (immunoturbidimetric method, Siemens INNOVANCE reagent), antithrombin (functional, Siemens INNOVANCE reagent), and anti-factor Xa activity (BioPhen Heparin LRT without antithrombin addition, lower limit of quantification 0.1 IU/L) were analyzed on Sysmex C5100 (Sysmex, Kobe, Japan). Markers of inflammation and organ dysfunction were analyzed on Siemens ADVIA Chemistry XPT or ADVIA Centaur XPT (Siemens Healthineers, Erlangen, Germany).

### Data Management and Statistics

Study data were managed using REDCap electronic data capture tools hosted at Aarhus University, Denmark.[24] Statistical analysis was performed in Stata version 17.0 (StataCorp, TX), and graphs were created using GraphPad, Prism 9 (GraphPad Software Inc., CA).

Normal distribution was assessed with quantile–quantile plots, but all data are presented as median with interquartile range (IQR) for uniformity, as not all variables followed normal distribution. Differences in hemostasis parameters between day 1, 3–4, and 7–8 were assessed with repeated measures analysis of variance (ANOVA) or Friedman's test. Differences between COVID-19 patients and healthy controls, between patients with and without thrombosis or with and without bleeding, and between survivors and nonsurvivors were assessed with *t*-test (with Welch's correction in case of unequal standard deviations [SDs]), or with Mann–Whitney test, when appropriate. Distribution of categorical variables between groups (i.e., presence or absence of lysis-resistant curve in patients with thrombosis versus no thrombosis) was assessed with Fisher's exact test. Correlation between continuous variables was assessed with Spearman's test.

In the statistical analyses of platelet aggregation measures, we excluded patients receiving aspirin when the agonist was AA and excluded patients receiving clopidogrel when ADP was the agonist. We also excluded patients with a history of thrombosis prior to the ICU admission (but during the COVID-19 admission) when the outcome of interest was thrombosis, and similarly excluded patients with a history of bleeding prior to the ICU admission when the outcome of interest was bleeding. Coagulation parameters of ECMO patients are reported separately as the hemostasis of these patients differs substantially from non-ECMO patients due to continuous infusion of unfractionated heparin.

We aimed to include the largest number possible of COVID-19 patients admitted to the ICU during 10 months. Therefore, no formal sample size calculation was performed. We performed posthoc power calculations for the two outcomes' difference in MCF and in ETP between COVID-19 patients on day 1 and healthy controls. For ROTEM MCF, mean with SD was 73 (SD: 5.2) mm for 42 COVID-19 patients and 63 (4.4) mm for 50 controls; for ETP, mean with SD was 1,129 (SD: 494) for 38 COVID-19 patients and 1,281 (208) for 90 controls. This yielded a study power to detect a difference between groups of $>0.99$ for EXTEM MCF and 0.43 for ETP.

### Systematic Review of Published Studies Applying Dynamic Assays in ICU COVID-19 Patients

#### Literature Search

PubMed and Embase were searched on April 14, 2021, with an update on June 22 2021, limited to publications from 2020 or 2021. Relevant MeSH/Emtree terms were employed and free-text words were added to include studies with related indexing. The search strings were constructed to include all studies on patients with COVID-19, which could contain data regarding coagulation or fibrinolysis from dynamic assays or dynamic and conventional assays in combination. Hence, the following terms were included in the search strings: COVID-19 OR coronavirus OR SARS-CoV-2 AND platelet function tests OR platelet activation OR platelet aggregometry OR impedance aggregometry OR light aggregometry OR platelet function analysis OR point-of-care testing OR platelet activation OR platelet function OR platelet aggregation OR thromboelastography OR thromboelastometry OR blood coagulation tests OR partial thromboplastin time OR international normalized ratio OR prothrombin time OR coagulation OR thrombin OR fibrinolysis OR hypofibrinolysis OR hyperfibrinolysis OR fibrin clot lysis time. No filters were set apart from date.

#### Inclusion and Exclusion Criteria of Overview of Previously Published Studies

Original papers fulfilling the following criteria were included: (1) patients admitted to ICU due to COVID-19, (2) applying platelet aggregation, dynamic coagulation, and/or fibrinolysis assay, (3) prospective data collection, (4) $\geq$20 cases, (5) $\geq$18 years of age, (6) a control group or reference range was provided, and (7) English. The exclusion criteria were: (1) retrospective data collection, (2) reviews, letters-to-the-editor, expert opinion, guidelines, etc. without original data, (3) conference abstract, and (4) studies including animal or in vitro data only.

After omission of duplicates, two authors (C.L.H. and J.B.L.) screened 50 publications by title and abstract. The remaining abstracts were then screened by either C.L.H. or J.B.L. Studies considered eligible or possibly eligible were assessed in full text and evaluated by the authors C.L.H., J.B.L., and A-.M.H. Any disagreement was solved between the authors by discussion until consensus was reached.

#### Data Extraction

Data extraction was performed by C.L.H. and verified by J.B.L. Outcomes were divided into biochemical outcomes, defined as laboratory results, and clinical outcomes, defined as occurrence of bleeding, thrombosis, or death.

## Results

### Cohort Study

Between April 2020 and February 2021, 81 patients were screened for eligibility and 51 patients were included in the study (►**Fig. 1**). Thirty-two patients were still in the ICU for the day 7–8 blood sample, and none was lost to follow-up.

 

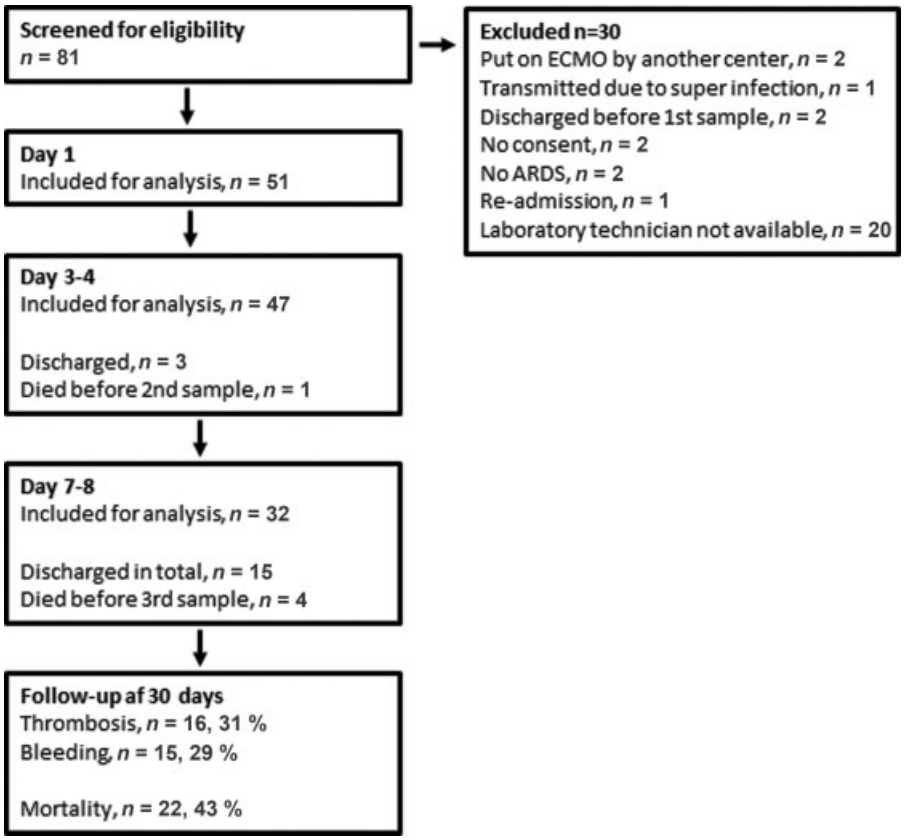


**Fig. 1** Flow chart for the inclusion of intensive care unit COVID-19 patients in the cohort study.

Demographic, baseline clinical data, and outcome data are provided in ►**Tables 1** and **2**. Median age was 67 years and 29% were female. Median BMI was 28 kg/m$^2$ and the most prevalent comorbidity was hypertension (57%), while 22% had chronic pulmonary disease, and 18% had chronic renal disease. The median SOFA score was 5 on day 1, while the median ISTH DIC score was 2. These scores remained unchanged during the study period.

Generally, patients received LMWH in standard prophylactic to intermediate dosing when admitted to the ICU, unless they received ECMO and therefore received unfractionated heparin infusion (►**Table 2**). Further, ECMO patients received antiplatelet therapy and antithrombin concentrate to maintain an antithrombin level above 0.80 IU/L relative to normal. In patients not on ECMO, antiplatelet therapy was only administered if the patient was already receiving this treatment prior to admission. One patient had received rivaroxaban within 2 days of inclusion. None of the patients received tranexamic acid and none received parenteral nutrition during the study period. Baseline biochemical characteristics are presented in ►**Table 3**, revealing an inflammatory response with increased C-reactive protein, white blood cell count, and ferritin, as well as severely increased fibrinogen and moderately increased fibrin D-dimer.

### Primary Hemostasis

Median values for platelet parameters are displayed in ►**Table 4**. The median platelet count, immature platelet count and fraction, and MPV were all within reference range on day 1. Platelet aggregation evaluated by impedance aggregometry and light transmission aggregometry revealed significantly reduced aggregation compared with healthy controls on day 1. However, measurements by light transmission aggregometry were within the in-house established reference interval.

Median platelet count, immature platelet count and fraction, as well as MPV increased over time. All remained within reference intervals, except for MPV, which was slightly above reference range on day 7–8.

AUC for impedance aggregometry increased from day 1 to 7–8 and became equivalent to the healthy controls. Maximal aggregation measured by light transmission aggregometry did not change during the study period.

### Global Whole Blood Coagulation Assay—ROTEM

ROTEM parameters are displayed in ►**Table 5**. Compared with healthy controls, both EXTEM and INTEM clotting times were prolonged at day 1, while the t-MaxVel was equivalent to healthy controls. MaxVel was increased in comparison to the control group. MCF of EXTEM, INTEM, and FIBTEM was higher among COVID-19 patients than in healthy controls. On day 1, INTEM maximum lysis was lower in ICU COVID-19 patients than in healthy controls, while EXTEM maximum lysis was equivalent between the two groups. Over time, all ROTEM parameters remained stable, except for maximum lysis in all assays, which decreased significantly.

 

**Table 1** Demographic, comorbidity, and outcome data on 51 intensive care unit COVID-19 patients

| | |
|---|---|
| *Demography* | |
| Age, median (IQR) | 67 (56–73) |
| Female, *n* (%) | 15 (29%) |
| BMI (kg/m$^2$), median (IQR) | 28 (28–35) |
| SAPS 3 (0–217), median (IQR) | 54 (50–62) |
| Comorbidity and medical history | |
| Hypertension, *n* (%) | 29 (57%) |
| Previous VTE, *n* (%) | 4 (8%) |
| Anticoagulant treatment prior to admission (any reason), *n* (%) | 8 (16%) |
| Antiplatelet therapy prior to admission, *n* (%) | 12 (24%) |
| Admission to hospital due to spontaneous hemorrhage within 1 year prior to this admission, *n* (%) | 1 (2%) (GI channel) |
| Charlson comorbidity index, median (IQR) | 1 (0–2)<br>Range 0–6 |
| *Outcome* | |
| Thrombosis | |
| Patients with thrombosis from inclusion to day 30, *n* (%) | 16 (31%) |
| During the study period, day 1–7 | 10 |
| After the study period until day 30 | 6[a] |
| Venous thrombosis | 15 |
| Arterial thrombosis | 3[b] |
| Bleeding according to GUSTO score | |
| Total number of patients with bleeding from inclusion to day 30, *n* (%) | 15 (29%)[c] |
| Minor bleeding | 6 |
| Moderate or severe bleeding | 9 |
| Mortality | |
| Mortality, 30-day, *n* (%) | 22 (43%) |

Abbreviations: BMI, body mass index; GI, gastrointestinal; IQR, interquartile range; SAPS, Simplified Acute Physiology Score; VTE, venous thromboembolism.
[a]One of these patients experienced thrombosis both during and after the study period.
[b]Two of these patients suffered both arterial and venous thrombosis.
[c]Two of these patients experienced bleeding both during and after the study period.

### Ex Vivo and In Vivo Thrombin Generation

On day 1, both ETP and peak thrombin were comparable with healthy controls (►**Fig. 2** and ►**Table 6**). Clot initiation was delayed, illustrated by prolonged lag time and time to peak in comparison to the healthy controls. The in vivo thrombin generation, reflected by TAT complex and F1 + 2 levels, was increased in comparison to healthy controls (►**Fig. 2** and ►**Table 6**).

While lag time, time to peak, and peak remained stable during the study period, the ETP decreased significantly from day 1 to day 7–8. TAT complex levels decreased slightly over time, while the F1 + 2 concentration fluctuated (►**Fig. 2** and ►**Table 6**).

### Fibrinolysis

On day 1, median PAI-1 levels were within reference range, while PAP complex levels were above reference range (►**Table 6**). Levels of PAI-1 showed great dispersion and on admission, 12 patients had a PAI-1 level above the reference range.

In 18 out of 46 (39%) patients, flat clot lysis curves were displayed on day 1. The remaining clot lysis curves on day 1 were either normal (18/46, 39%) or lysis-resistant (10/46, 22%). Peak fibrin formation, AUC, and 50% lysis time were all significantly increased on day 1 (►**Fig. 3** and ►**Table 6**). Of the 12 patients with increased PAI-1 on admission, 7 patients had lysis resistant curves while 5 had flat curves.

Over time, the median PAI-1 level and the parameters of the clot lysis assay remained stable, while PAP complex levels decreased but still remained above the reference range (►**Table 6**).

### Anti-factor Xa Activity

On day 1, 63% of the ICU COVID-19 patients had an anti-factor Xa level above 0.10 IU/L (►**Table 6**). Over time, this number increased to 89%. There was a significant and moderate to

 

**Table 2** Clinical data on 51 intensive care unit COVID-19 patients

| | Day 1 ($n = 51$) | Day 3–4 ($n = 47$) | Day 7–8 ($n = 32$) |
|---|---|---|---|
| Clinical scores | | | |
| SOFA score (0–24), median (IQR) | 5 (4–8) | 5.5 (4–8) | 6 (3–9) |
| ISTH DIC score (0–8), median (IQR) | 2 (2–3) | 2 (2–2) | 2 (1.5–3) |
| Ventilator therapy | | | |
| High flow oxygen by nasal cannula | 19 | 14 | 4 |
| Intubated | 28 | 33 | 28 |
| VV ECMO | 4 | 4 | 4 |
| Dialysis | | | |
| CRRT | 2 | 5 | 4 |
| Intermittent hemodialysis | 1 | 1 | 0 |
| Anticoagulant therapy | | | |
| UFH infusion (ECMO) | 4 (700–2,200 IU/h) | 4 (2,300–2,400 IU/h) | 4 (1,200–2,500 IU/h) |
| LMWH (dalteparin), *n* (%) | 42 (82%) | 42 (89%) | 28 (82%) |
| Prophylactic dosing (2,500–5,000 IU/24 h) | 14 | 3 | 2 |
| Intermediate dosing (7,500–10,000 IU/24 h) | 21 | 24 | 15 |
| Therapeutic dosage (200 IU/kg/24 h) | 7 | 15 | 11 |
| No UFH or LMWH, *n* (%) | 5 (10%) | 1 (2%) | 0 (0%) |
| Platelet inhibitor within 5 days of blood sampling | 13 | 12 | 7 |
| ASA | 10 | 10 | 6 |
| Clopidogrel | 3 | 2 | 1 |
| Transfusion within 24 hours prior to blood sampling | | | |
| Red blood cells, *n* (median, range volume) | 6 (542 mL, range: 255–1,079 mL) | 2 (253 mL, range: 248–257 mL) | 4 (279 mL, range: 263–515 mL) |
| Fresh frozen plasma, *n* (median, volume) | 1 (307 mL) | 1 (300 mL) | 1 (765 mL) |
| Platelet concentrate, *n* (median, volume) | 0 | 0 | 1 (375 mL) |

Abbreviations: ASA, acetylsalicylic acid; CRRT, continuous renal replacement therapy; DIC, disseminated intravascular coagulation; ECMO, extracorporeal membrane oxygenation; ISTH, International Society of Thrombosis and Hemostasis; LMWH, low-molecular-weight heparin; SOFA, sequential organ failure assessment; UFH, unfractionated heparin; VV, venous to venous.

strong negative correlation between the ETP and the anti-factor Xa level (rho −0.56 to −0.81, $p < 0.001$ on all 3 days). Patients with flat clot lysis curves had an anti-factor Xa level of 0.30 (0.17–0.66) UI/L, while patients with normal or lysis-resistant clot lysis curves had an anti-factor Xa level of 0 (0–0.17) UI/L.

### Thrombosis

During the 30-day follow-up period, 16 (31%) patients had venous ($n = 15$) or arterial thrombosis ($n = 3$), with two patients suffering both arterial and venous thrombosis (►**Table 1**).

Patients diagnosed with thrombosis after study inclusion presented with higher in vivo thrombin generation, expressed by higher TAT complex levels and higher F1 + 2 concentrations on day 1 than patients not developing thrombosis (►**Fig. 2** and ►**Table 6**). This increase in thrombin generation was not reflected in the ex vivo thrombin generation parameters (lag time, time to peak, peak, ETP, all $p > 0.15$). Likewise, ROTEM parameters did not differ between the two groups (►**Table 5**).

Fibrinolysis parameters did not differ between patients developing thrombosis and those who did not develop thrombosis (►**Fig. 3**). We investigated the distribution of patients with normal and prolonged 50% lysis time (above our upper reference limit) on day 1 in the thrombosis versus no thrombosis group, after excluding patients with flat curves ($n = 18$). In the thrombosis group, 7/10 patients had prolonged 50% lysis time versus 10/17 in the no thrombosis group (70 vs. 59%; $p = 0.69$, Fisher's exact test). Likewise, the maximum lysis in ROTEM was equivalent in the group with thrombosis and the group not developing thrombosis (►**Table 5**).

 

**Table 3** Baseline biochemical characteristics of 51 intensive care unit COVID-19 patients at the date of study inclusion

| | Reference range | Median value (IQR) |
|---|---|---|
| Coagulation and hematology | | |
| INR | <1.2 | 1.1 (1.0–1.2) |
| aPTT, s | 20–29 | 25 (20–31)[a] |
| Antithrombin (activity), IU/L | 0.80–1.20 | 0.99 (0.84–1.15)[a] |
| Fibrinogen (functional), μmol/L | 5.5–12.0 | 20.5 (15.7–25.1)[a] |
| Fibrin D-dimer, mg/L (FEU) | <0.70 | 2.18 (0.85–4.37)[b] |
| White blood cells, $\times 10^9$/L | 3.5–10.0 | 9.5 (6.1–13.5) |
| Neutrophils, $\times 10^9$/L | 2.0–7.0 | 8.5 (5.3–12.6)[b] |
| Lymphocytes, $\times 10^9$/L | 1.3–3.5 | 0.7 (0.6–1.0)[b] |
| Hemoglobin, mmol/L | 7.3–10.5 | 7.2 (6.2–7.8)[b] |
| Inflammation | | |
| CRP, mg/L | <8 | 165 (82–274) |
| Procalcitonin, μg/L | <0.5 | 0.3 (0.2–1.1)[a] |
| Ferritin, μg/L | 15–355 | 1043 (559–1,515)[b] |
| Cardiac | | |
| Cardiac troponin I, ng/L | <47 | 17 (8–50)[a] |
| Creatine kinase MB, μg/L | <4.0 | 1.2 (0.7–2.5)[b] |
| Creatine kinase, μg/L | 50–270 | 117 (45–258)[b] |
| Pro-brain natriuretic peptide, ng/L | <300 | 538 (205–1,531)[b] |
| Liver | | |
| Triglyceride, mmol/L | <2.0 | 1.8 (1.4–2.4)[b] |
| Amylase, pancreas type, U/L | 10–65 | 39 (21–65)[a] |
| LDH, U/L | 105–255 | 410 (341–512)[a] |
| Alanine aminotransferase, U/L | 10–70 | 37 (24–57)[b] |
| Bilirubin, μmol/L | 5–25 | 10 (7–15) |
| Alkaline phosphatase, U/L | 35–105 | 75 (51–103)[a] |
| Renal | | |
| Potassium, mmol/L | 3.5–4.6 | 4.0 (3.8–4.2) |
| Sodium, mmol/L | 137–145 | 140 (137–145) |
| Calcium, mmol/L | 2.20–2.55 | 2.22 (2.16–2.30)[b] |
| Albumin, g/L | 34–48 | 26 (23–28) |
| Creatinine, μmol/L | 45–105 | 98 (75–151) |
| Urea, mmol/L | 2.6–8.1 | 12.7 (9.3–19.1)[b] |
| eGFR/1.73 $m^2$ (CKD-EPI), mL/min | >60 | 66 (40–87) |

Abbreviations: aPTT, activated partial thromboplastin time; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; INR, International normalized ratio; LDH, lactate dehydrogenase; MB, myocardial band.
Note: Reference ranges for fibrin D-dimer, hemoglobin, ferritin, creatine kinase, albumin, creatinine, and urea combined for sex and across age.
[a]3–4 missing values.
[b]1–2 missing values.

### Bleeding

Fifteen patients experienced episodes of bleeding from inclusion and until day 30 (►**Table 1**). Episodes were minor ($n = 6$) to moderate ($n = 8$), and one patient experienced severe hemorrhage requiring intervention (coil procedure). Three of the six patients receiving red blood cells on day 1 were transfused due to start-up of ECMO treatment.

Between the groups of moderate to severe bleeding ($n = 9$, 22%) and non/minor bleeding patients ($n = 32$, 78%), there were no systematic differences in aggregation, coagulation, or fibrinolysis parameters. Of note, platelet aggregation measures

 

**Table 4** Platelet count and aggregation results from 47 intensive care unit COVID-19 patients compared with healthy controls (extracorporeal membrane oxygenation [ECMO] patients ($n = 4$) are excluded from this analysis)

| | | Development over time | | | Comparison between patients with and without thrombosis, day 1 sample | | |
|---|---|---|---|---|---|---|---|
| | Healthy controls | Day 1, median (IQR) | Day 3–4, median (IQR) | Day 7–8, median (IQR) | Patients with thrombosis | Patients without thrombosis | *p*-Value |
| Platelet counts | Reference range | $n = 47$ | $n = 43$ | $n = 29$ | $n = 16$ | $n = 31$ | |
| Platelet count, $\times 10^9$/L | 145–400 | 273 (198–357) | 331 (251–427)[a] | 343 (256–412)[b] | 310 (216–379)[a] | 235 (176–318)[a] | 0.10[c] |
| Mean platelet volume (MPV), fL | 6.5–11.0 | 10.4 (10.0–11.2)[d] | 10.6 (10.0–11.3)[e] | 11.2 (10.7–12.0)[a,b] | 10.2 (9.6–11.2)[d] | 10.4 (10–11.1)[d] | 0.71[c] |
| Immature platelet count, $\times 10^9$/L | 4.4–26.7 | 11.1 (8.0–17.1)[d] | 16.2 (9.9–22.1)[d] | 18.2 (14.4–30.4)[f] | 12.7 (6.7–18.2)[d] | 9.8 (8.0–11.8)[d] | 0.70[g] |
| Immature platelet fraction | 0.016–0.126 | 0.046 (0.032–0.059)[d] | 0.055 (0.034–0.067)[d] | 0.071 (0.044–0.096)[f] | 0.042 (0.024–0.063)[d] | 0.047 (0.032–0.065)[d] | 0.36[g] |
| Light transmission aggregometry (PAP8) | Reference range | | | | | | |
| ADP, maximal aggregation, % | 64–105 | 73 (62–76)[d] | 65 (58–73)[e] | 67 (59–74)[d] | 71 (54–74)[a] | 73 (69–83)[d] | 0.20[g] |
| Collagen, maximal aggregation, % | 51–92 | 73 (64–79)[d] | 74 (62–79)[e] | 72 (66–76)[a] | 73 (69–82)[a] | 73 (64–78)[d] | 0.67[g] |
| AA, maximal aggregation, % | 60–95 | 26 (15–43)[d] | 39 (16–55)[e] | 39 (14–50)[d] | 26 (11–39)[d] | 27 (16–44)[e] | 0.45[c] |
| Impedance aggregometry (Multiplate) | Median (IQR) | | | | | | |
| ADP, area under the curve, AU*min | 782 (738–946) | 587 (393–788)[e] | 535 (330–755)[e] | 627 (400–848)[d] | 440 (366–753)[a] | 599 (421–769)[d] | 0.50[c] |
| Collagen, area under the curve, AU*min | 464 (392–598) | 385 (258–532)[d] | 463 (346–614)[e] | 452 (311–526)[a] | 324 (204–378)[a] | 401 (295–532)[d] | 0.39[c] |
| AA, area under the curve, AU*min | 1066 (957–1,126) | 725 (487–1,005)[e] | 755 (561–1,087)[e] | 1053 (875–1,428)[d] | 504 (335–767)[d] | 888 (595–973)[e] | 0.09[c] |

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; IQR, interquartile range.
Note: Significant difference over time, $p < 0.05$. *p*-Values are from tests between patients with and without thrombosis from study inclusion and until day 30.
[a]1–3 missing.
[b]Repeated measurement ANOVA.
[c]*t*-Test.
[d]4–7 missing.
[e]8–10 missing.
[f]Friedman's test.
[g]Mann–Whitney test.

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**Table 5** Rotational thromboelastometry (ROTEM) results from 47 intensive care unit COVID-19 patients compared with healthy controls (extracorporeal membrane oxygenation [ECMO] patients are excluded from this analysis)

| | | Development over time | | | Comparison between patients with and without thrombosis, day 1 sample | | |
|---|---|---|---|---|---|---|---|
| | Healthy controls | Day 1, median (IQR) | Day 3–4, median (IQR) | Day 7–8, median (IQR) | Patients with thrombosis | Patients without thrombosis | *p*-Value |
| ROTEM EXTEM | Median (IQR) | *n* = 42 | *n* = 31 | *n* = 26 | *n* = 13 | *n* = 26 | |
| Clotting time (s) | 54 (50–60) | 75 (65–83) | 70 (62–75) | 67 (59–75) | 75 (65–75) | 74 (65–84) | 0.29[a] |
| MaxVel (mm/min) | 15 (13–17) | 25 (21–30) | 29 (24–31) | 27 (22–31) | 24 (20–28) | 25 (22–29) | 0.62[a] |
| t-MaxVel (s) | 101 (88–120) | 102 (94–121) | 103 (85–122) | 107 (100–115) | 103 (97–121) | 100 (85–121) | 0.64[a] |
| MCF (mm) | 57 (54–61) | 74 (69–76) | 74 (69–76) | 75 (72–77) | 73 (69–75) | 74 (69–76) | 0.96[a] |
| ML (%) | – | 6 (4–9) | 7 (3–9) | 4 (3–6)[b] | 5 (4–7) | 7 (4–9) | 0.35[a] |
| ROTEM INTEM | | | | | | | |
| Clotting time (s) | 156 (144–163) | 178 (151–204) | 175 (161–204) | 175 (150–192) | 151 (144–219) | 181 (162–202) | 0.31[c] |
| MaxVel (mm/min) | 18 (16–20) | 27 (22–33) | 30 (25–36) | 30 (26–35) | 24 (22–29) | 28 (22–32) | 0.61[a] |
| t-MaxVel (s) | 181 (172–199) | 214 (183–234) | 212 (188–235) | 211 (190–223) | 195 (178–258) | 216 (193–229) | 0.46[a] |
| MCF (mm) | 61 (57–63) | 74 (68–76) | 75 (69–78) | 76 (74–79) | 72 (70–75) | 73 (68–77) | 0.99[a] |
| ML (%) | – | 5 (2–9) | 5 (2–9) | 2 (1–4)[b] | 4 (3–7) | 6 (2–9) | 0.47[a] |
| ROTEM FIBTEM | | | | | | | |
| MCF (mm) | 14 (12–15) | 38 (34–43) | 39 (32–44) | 38 (30–43) | 38 (33–41) | 38 (34–43) | 0.81[a] |

Abbreviations: IQR, interquartile range; MaxVel, maximum velocity; t-MaxVel, time to maximum velocity; MCF, maximum clot firmness; ML, maximum lysis; ROTEM, rotational thromboelastometry.
Note: *p*-Values are from tests between patients with and without thrombosis from study inclusion and until day 30.
[a]*t*-Test.
[b]Significant difference over time: Friedman's test.
[c]Mann–Whitney test.

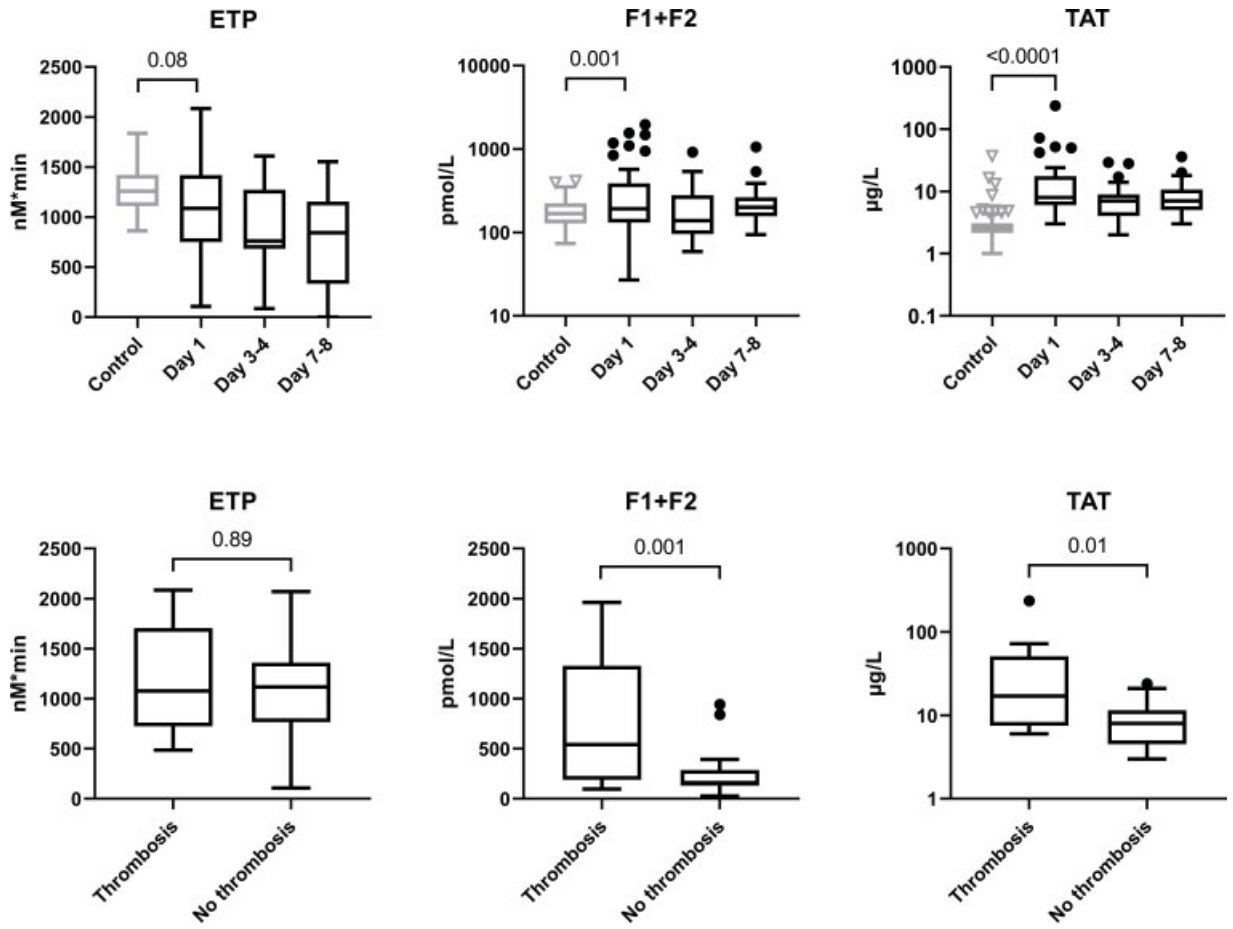


**Fig. 2** Ex vivo and in vivo thrombin generation in intensive care unit COVID-19 patients. Top: in relation to healthy controls and development over time. Bottom: divided into groups according to occurrence of thrombosis within 30 days of inclusion.

did not differ between bleeding and non/minor bleeding patients (data not shown). Only INTEM MCF was increased (median (IQR): 76 (73–82) vs. 71 (68–76) mm, $p = 0.01$), while INTEM maximum lysis was lower in the bleeding group than in the non/minor bleeding group ($p < 0.01$, data not shown).

### Mortality

The 30-day mortality was 43%, as 22/51 patients died within 30 days of study inclusion. The measured parameters were equivalent in dead versus alive patients on day 30 (all $p > 0.12$).

### ECMO Patients

In all, five patients received ECMO treatment. In one patient, treatment was started on day 7, and the data from this patient are evaluated with the data from patients not receiving ECMO treatment. In the remaining four patients, ECMO treatment had begun right before the first blood sample. Due to continuous heparin infusion, few ROTEM, ex vivo thrombin generation, and clot lysis assay data were available. On day 1, comparing ECMO patients with other ICU COVID-19 patients, median (IQR) TAT complex levels (15.7 (14.1–18.7) vs. 8.4 (6.2–19.5) µg/mL), F1 + 2 concentrations (820 (600–1,208) vs. 198 (132–386) ng/mL), PAI-1 (24.4 (4.7–52.6) vs. 18.1 (12.4–49.1) ng/mL) as well as PAP complex levels (2,265 (1,344–6,268) vs. 830 (530–1,224) ng/mL) were higher (no statistical tests performed due to low numbers). One ECMO patient suffered minor bleeding and one patient developed pulmonary thrombosis.

## Systematic Review

In total, 3,657 studies were identified after duplicate screening. Of these, 107 studies were evaluated by full-text reading and finally 18 were included in this review (►**Fig. 4**). The data extracted are presented in ►**Tables 7** to **9**.

Two studies applied more than one dynamic assay,[7,25] while one study applied only platelet aggregation measurements,[8] 10 presented ROTEM[2,9,26–30] or TEG data[31–33] and five studies reported thrombin generation measurements.[3,34–37] Two studies investigated fibrinolysis with the addition of tPa to their assay.[7,25] The studies included 20 to 48 ICU COVID-19 patients. Six (33%) studies related their biochemical findings to clinical outcome, either development of thrombosis, bleeding, or mortality.[3,25–27,31,32]

### Platelet Aggregation

Three studies investigated platelet aggregation, either by Multiplate,[7] the Total Thrombus-Formation Analysis System,[8] or ROTEM platelet[9] (►**Tables 7** and **8**). Aggregation measures were in all studies either within or below reference ranges. Ghirardello et al investigated the association between aggregation measurements and severity of the COVID-19, but found no association.[8]

 

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**Table 6** Ex vivo and in vivo thrombin generation parameters, ex vivo and in vivo clot lysis assay parameters, and anti-factor Xa measurements from intensive care unit COVID-19 patients

| | Development over time | | | | | Comparison between patients with and without thrombosis, day 1 sample | | |
|---|---|---|---|---|---|---|---|---|
| | Healthy, median (IQR) | Day 1, median (IQR) | Day 3–4, median (IQR) | Day 7–8, median (IQR) | *p*-Value | Patients with thrombosis | Patients without thrombosis | *p*-Value |
| Ex vivo thrombin generation Thrombin generation assay | $n = 90$ | $n = 38$ | $n = 23$ | $n = 22$ | | $n = 12$ | $n = 23$ | |
| Lag time (min) | 3.3 (3.0–3.8) | 5.5 (5.0–7.3) | 6.0 (4.3–8.9) | 6.5 (4.7–8.7) | 0.11 | 5.3 (4.2–6.8) | 5.7 (5.0–7.3) | 0.28 |
| Peak (nM) | 202 (166–234) | 179 (102–234) | 149 (91–248) | 133 (50–216) | 0.26 | 220 (117–282) | 174 (112–220) | 0.31 |
| Time to peak (s) | 7.2 (6.1–8.0) | 9.5 (7.8–12.5) | 9.5 (7.5–12.9) | 10.1 (8.2–12.7) | 0.12 | 8.3 (6.6–10.7) | 9.7 (8.0–12.5) | 0.15 |
| ETP (nM*min) | 1,259 (1,113–1,418) | 1,086 (758–1,398) | 762 (683–1,274) | 844 (340–1,145) | 0.01 | 1,077 (722–1,665) | 1,154 (780–1,363) | 0.89 |
| In vivo thrombin generation parameters | | $n = 43$ | $n = 32$ | $n = 28$ | | $n = 13$ | $n = 27$ | |
| TAT complex (μg/L) | 2.5 (2.2–3.1) $n = 122$ | 8.4 (6.2–19.5) | 6.8 (4.7–11.0) | 6.9 (4.8–10.8) | 0.02 | 16.6 (8.7–50.0) | 7.7 (4.6–12.7) | 0.01 |
| F1 + F2 (pmol/L) | 171 (130–226) $n = 95$ | 198 (132–386) | 147 (101–265) | 201 (160–263) | 0.02 | 541 (198–1,177) | 160 (129–301) | 0.001 |
| Ex vivo fibrinolysis Clot lysis assay | $n = 120$ | $n = 27$ | $n = 14$ | $n = 13$ | | $n = 10$ | $n = 16$ | |
| Peak (AU) | 0.46 (0.36–0.55) | 1.04 (0.90–1.12) | 1.00 (0.90–1.14) | 0.89 (0.58–1.03) | 0.08 | 1.05 (0.92–1.12) | 1.03 (0.88–1.13) | 0.71 |
| AUC (AU*s) | 463 (353–649) | 2,629 (1,747–3,260) | 2,626 (1,810–3,421) | 1,980 (1,446–2,573) | 0.56 | 2,594 (1,780–3,603) | 2,752 (1,538–3,253) | 0.67 |
| 50% lysis time (s) | 652 (532–875) | 2,125 (1,281–3,000) | 1,717 (1,191–3,000) | 1,551 (990–3,000) | 0.24 | 2,172 (1,384–3,000) | 2,178 (1,153–3,000) | 0.59 |
| In vivo fibrinolysis parameters | Reference range | $n = 43$ | $n = 32$ | $n = 29$ | | $n = 13$ | $n = 27$ | |
| PAI-1 (ng/mL) | 7–43 | 18.1 (12.4–49.1) | 16.4 (8.7–24.1) | 22.2 (11.7–40.4) | 0.09 | 16.7 (13.8–37.5) | 19.8 (12.2–57.3) | 0.75 |
| PAP complex (ng/mL) | <514 | 830 (530–1,224) | 675 (473–968) | 739 (292–1,073) | 0.0007 | 916 (730–2,152) | 794 (494–1,029) | 0.20 |
| Anti-factor Xa activity | | $n = 44$ | $n = 31$ | $n = 28$ | | | | |
| Anti-factor Xa activity, IU/L; median (IQR) | | 0.15 (0–0.31) | 0.33 (0.16–0.47) | 0.36 (0.2–0.36) | 0.06 | 0.06 (0.00–0.23) | 0.17 (0.00–0.44) | 0.18 |
| Anti-factor Xa ≥ 0.1 IU/L, *n* (%) | | 28/44 (63%) | 28/31 (90%) | 25/28 (89%) | | | | |

Abbreviations: AUC, area under the curve; ETP, endogenous thrombin potential; F1 + F2: prothrombin fragment 1 + 2; IQR, interquartile range; PAI-1, plasminogen activator inhibitor-1; PAP, plasmin–antiplasmin complex.

Note: Extracorporeal membrane oxygenation (ECMO) patients ($n = 4$) are excluded from this analysis. Flat curves ($n = 18$) are excluded from analysis and lysis-resistant curves are set to 50% lysis time, 3000 seconds.

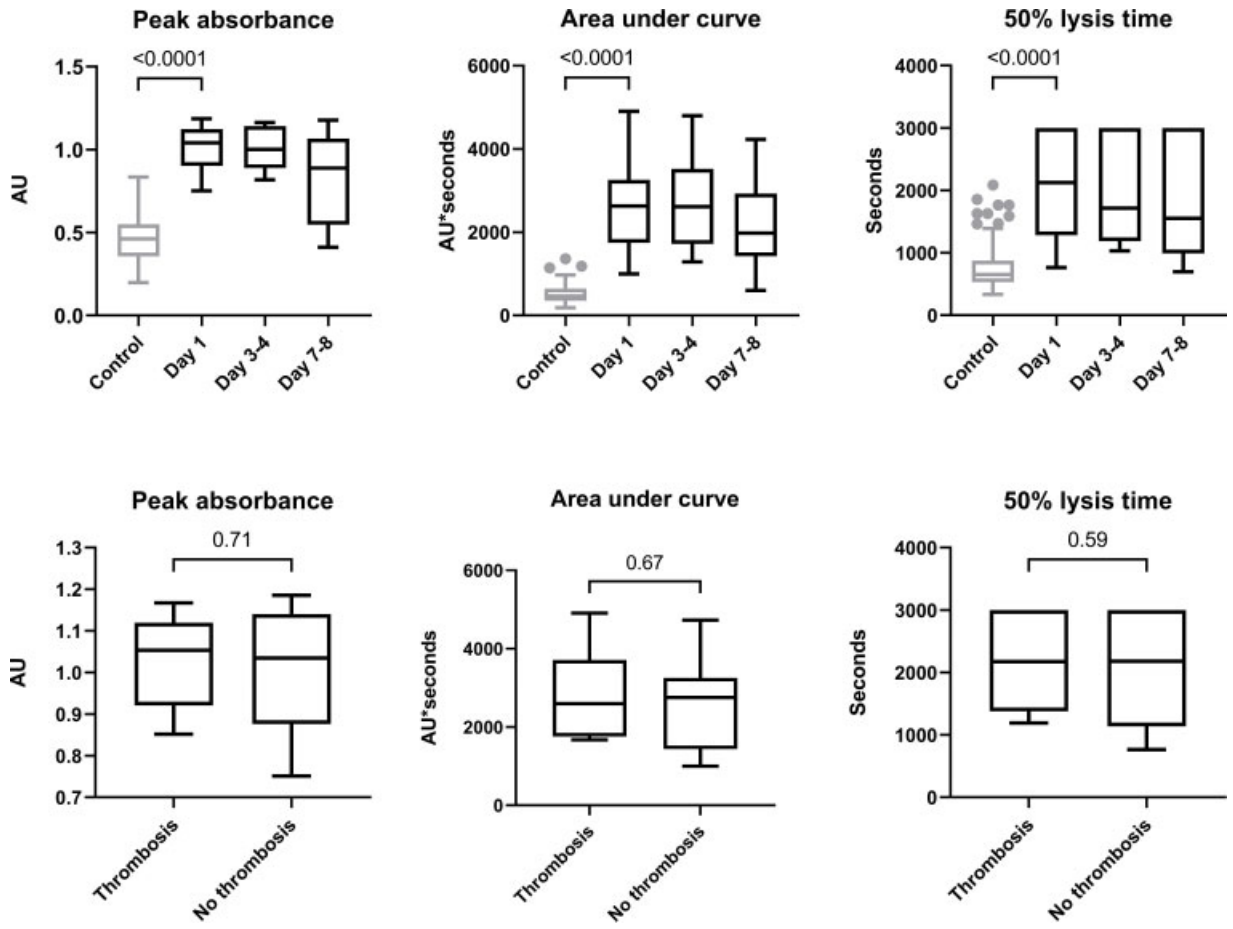


**Fig. 3** Clot lysis parameters in intensive care unit COVID-19 patients. Top: in relation to healthy controls and development over time. Bottom: divided into groups according to occurrence of thrombosis within 30 days of inclusion.

### Secondary Hemostasis Evaluated by Global Whole Blood Coagulation or Thrombin Generation Assays

Of the included studies, 11 studies applied the global whole blood coagulation assays ROTEM, TEG, TEG6, or ClotPro in their investigation of ICU COVID-19 patients[2,7,9,26–33] (►**Table 8**), while six studies applied thrombin generation assays[3,25,34–37] (►**Table 9**).

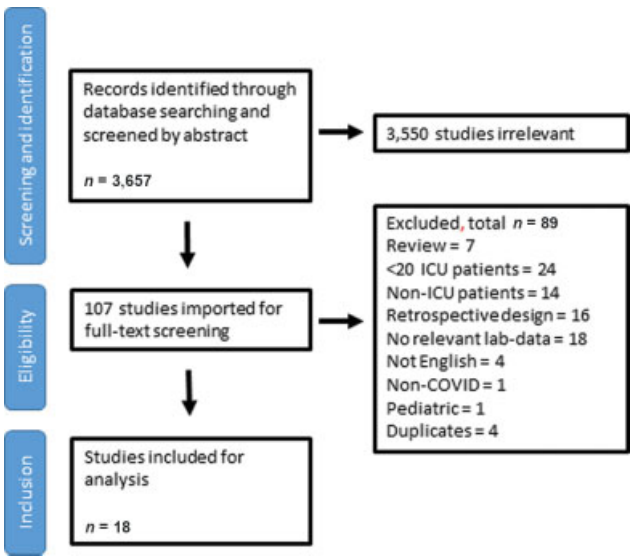


**Fig. 4** Flow chart for the inclusion of studies in the systematic review.

In the global whole blood coagulation assays, clotting times were prolonged[2,7,26,28] or normal,[9] except in the two studies applying kaolin-dependent assays, in which clotting time was shortened.[32,33] MCF/maximum amplitude was increased in the fibrinogen-dependent assays (FIBTEM, functional fibrinogen),[2,7,9,26–31] while MCF/maximum amplitude was increased[2,7,9,27,29,30] or normal[26,28,31] in the TF-dependent assays (EXTEM, Rapid MA).

Thrombin generation assays showed variable results but revealed normal[34] to prolonged lag time.[3,35,37] The ETP was comparable to healthy controls[25,34,35] or within reference range,[37] while when compared with non-ICU COVID-19 patients it was found to be increased[36] or even reduced.[3] However, the latter two studies differed in timing of blood sampling, as Canzano et al obtained blood samples on hospital admission, while de la Morena-Barrio et al sampled within median 2 (IQR: 1–7) days following admission. Moreover, the assay in the study by de la Morena-Barrio contained thrombomodulin, which was not the case in the study by Canzano et al.

### Fibrinolysis

Two studies investigated fibrinolysis, either by a modified ROTEM assay with tPa addition or the ClotPro, also adding

 

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**Table 7** Studies included in the systematic review applying dynamic platelet aggregation analysis, $n = 2$

| Author Publication year and month Publication type | Study characteristics Number of participants Control group | Timing of blood sampling Measurements of hemostasis (reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| Ghirardello et al[8]<br>Feb 2021<br>Article | 24 COVID-ICU<br>21 COVID sub-intensive care<br>16 COVID low intensive care<br>32 healthy controls | On enrollment and 7 days later<br>Divided according to time of enrollment:<br>A: within 8 days of admission<br>B: between day 9 and 21<br>C: >21 days after admission, median (range): 17 (2–59)<br>*Total thrombus-formation analysis System T-TAS:*<br>Occlusion time<br>Occlusion start time<br>AUC | *Group A vs. B vs. C vs. healthy, median (min–max):*<br>Occlusion time: ↑A 10.0 (4.4–11.0) vs. B 4.9 (2.5–11.0) vs. C 5.9 (2.4–11.0) vs. 4.5 (2.0–6.5) min, $p = 0.002$<br>AUC: ↓A 263 (13–410) vs. B 390 (108–491) vs. C 349 (23–500) vs. 388 (31–462) min*kPa, $p = 0.014$<br>→ Occlusion start time: A 2.1 (1.1–11) vs. B 1.8 (1.1–3.9) vs. C 2.0 (1.1–8.4) vs. 1.6 (1.1–2.6) min, $p = 0.909$<br>No difference between severity groups in any parameter<br>Change from enrollment to day 7, nonsignificant for all parameters | *VTE, in-hospital: 12 (19%)*<br>*Mortality within 7 days of enrollment:*<br>6/61 (9.9%) |
| Heinz et al[7]<br>Jan 2021<br>Article<br>ClotPro results presented in ►**Table 8** | 27 COVID-ICU<br>12 healthy controls | Mean ICU stay from admission to sample 7 ± 3.5 days<br>*Multiplate:*<br>Agonists ADP, AA, and TRAP | *Multiplate, COVID-ICU vs. healthy, mean ± SD:*<br>↓AUC for ADP 68 ± 37 vs. 91 ± 29 U, $p = 0.043$<br>AUC not different for AA or TRAP<br>12 COVID-ICU patients had AUC for ADP below reference range | No clinical outcome measures |

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; AUC, area under the curve; COVID, corona virus disease; ICU, intensive care unit; NA, not available; PPP, platelet-poor plasma; SD, standard deviation; TRAP, thrombin receptor activating peptide; VTE, venous thrombus embolism.

**Table 8** Studies included in the systematic review applying global whole blood coagulation assays (ROTEM, TEG, ClotPro), $n = 12$

| Author<br>Publication year and month<br>Publication type | Study characteristics<br>Number of participants<br>Control group | Timing of blood sampling<br>Measurements of hemostasis (reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| Almskog et al[2]<br>Oct 2020<br>Article | 20 COVID-ICU<br>40 COVID regular ward<br>86 healthy controls | Median within 1 day of admission<br>*ROTEM:*<br>EXTEM, INTEM, FIBTEM, HEPTEM | *COVID-ICU vs. healthy, median (IQR):*<br>EXTEM<br>↑ CT 90 (78–108) vs. 47 (43–51), $p < 0.001$<br>↓ CFT 46 (42–55) vs. 91 (79–101), $p < 0.001$<br>↑ MCF 76 (71–77) vs. 64 (62–68) $p < 0.001$<br>INTEM<br>↑ CT 183 (175–195) vs. 163 (157–170), $p < 0.001$<br>↓ CFT 46 (40–59) vs. 63 (57–72) $p = 0.001$<br>FIBTEM<br>↑ MCF 32 (28–36) vs. 13 (11–15), $p < 0.001$ | No outcome measures |
| Bocci et al[31]<br>Dec 2020<br>Article | 40 COVID-ICU | Within 24 hours of admission to ICU and 7 days later<br>*TEG6:*<br>Kaolin:<br>R-time, min (4.6–9.1)<br>Rapid (Kaolin + tissue factor): R-time (0.3–1.1 min)<br>MA (52–70 mm)<br>functional fibrinogen (tissue factor + platelet inhibition):<br>MA (15–32 mm)<br>Kaolin + heparinase | *COVID-ICU, median (IQR) vs. reference range:*<br>→ Kaolin-R-time 7.1 (5.2–8.1) min<br>→ Rapid-R-time 0.3 (0.2–0.4) min<br>→ Rapid-MA 69.8 (66.3–71.3) mm<br>↑ FF-MA 42.2 (30.9–49.2) mm<br>Seven-day parameters not different from admission samples | *Thrombosis, within 28 days:*<br>2/40 (0.05%)—both PE<br>*28 day mortality:*<br>17/40 (42.5%)<br>No difference in any TEG6 parameter between dead or alive patients<br>*Alive vs. dead, median (IQR):*<br>→ Kaolin-R-time 6.9 (5.2–7.8) vs. 7.8 (5–8.3), $p = 0.40$<br>→ Rapid-R-time 0.4 (0.2–0.04) vs. 0.3 (0.2–0.4), $p = 0.20$<br>→ Rapid-MA 68.8 (66.2–71.8) vs. 70.3 (68.9–71), $p = 0.97$<br>→ FF-MA 39.1 (29.9–49.2) vs. 43.7 (36.4–48.7), $p = 0.44$ |
| Boscolo et al[26]<br>July 2020<br>Letter | 32 COVID-ICU<br>32 COVID<br>Internal medical ward | Timing not stated<br>*ROTEM:*<br>EXTEM, INTEM, FIBTEM<br>Reference ranges not provided | *COVID-ICU vs. COVID medical ward, median (IQR):*<br>↑ FIBTEM MCF 33 (27–41) vs. 30 (25–33) mm, $p = 0.02$<br>↑ EXTEM CT 74 (64–88) vs. 65 (61–72) s, $p < 0.01$<br>↑ EXTEM CFT 60 (48–80) vs. 43 (38–56) s, $p < 0.01$<br>No significant difference in: INTEM/EXTEM MCF, INTEM CT, INTEM CFT between COVID-ICU and COVID medical ward | *Thrombosis, COVID-ICU vs. medical ward:*<br>11 (34%) vs. 3 (9%), $p = 0.03$<br>ROTEM parameters not related to risk of thrombosis or mortality<br>*28-day mortality, COVID-ICU vs. medical ward:*<br>8 (25%) vs. 1(3%), $p = 0.03$ |
| Corrêa et al[9]<br>Dec 2020<br>Article | 30 COVID-ICU | Day 1, 3, 7, 14 after admission to ICU<br>*ROTEM:*<br>EXTEM, INTEM, FIBTEM<br>*ROTEM platelet:* | *Admission, COVID-ICU vs. reference range, median (IQR):*<br>↑ EXTEM MCF 73 (69–74) vs. 50–72 mm<br>↑ FIBTEM MCF 36 (32–38) vs. 9–25 mm<br>*On admission, within reference range:*<br>INTEM CT, CFT, MCF, ML<br>EXTEM CT, CFT, ML | *Thrombosis, during ICU stay:*<br>6/30 (20%), 4 DVT, 2 PE<br>*Bleeding, during ICU stay:*<br>3/30 (10%) |

(*Continued*)

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**Table 8** (*Continued*)

| Author<br>Publication year and month<br>Publication type | Study characteristics<br>Number of participants<br>Control group | Timing of blood sampling<br>Measurements of hemostasis (reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| | | ARATEM (70–153 s)<br>ADPTEM (56–139 s) | ARATEM, ADPTEM<br>*Over time, from admission to day 14:*<br>EXTEM:<br>(↑) CT 72 (66–79) to 72 (61–80) s, $p = 0.013$<br>(↑) MCF 73 (69–74) to 74 (69–79) mm, $p < 0.001$<br>↓ ML 10 (7–12) to 7 (5–11)%, $p = 0.006$<br>INTEM:<br>↑ MCF 70 (67–72) to 76 (69–79) mm, $p < 0.001$<br>↓ ML 10 (6–12) to 4 (2–6)%, $p < 0.001$<br>↑ ARATEM 79 (54–110) to 110 (83–154) s, $p = 0.014$<br>↑ ADPTEM 96 (65–111) to 112 (67–134) s, $p = 0.004$ | |
| Heinz et al[7]<br>Jan 2021<br>Article<br>Platelet aggregation results presented in ►**Table 7** | 27 COVID-ICU<br>12 healthy controls | Mean ICU stay from admission to sample 7 ± 3.5 days<br>*ClotPro:*<br>Extrinsic activation<br>Intrinsic activation<br>Contribution of fibrin<br>Extrinsic + addition of tPa | *ClotPro, COVID-ICU vs. healthy, mean ± SD:*<br>Extrinsic activation:<br>↑ CT 88 ± 22 vs. 60 ± 7 s, $p < 0.001$<br>↑ MCF 68 ± 5 vs. 57 ± 4 mm, $p < 0.001$<br>Intrinsic activation:<br>↑ CT 262 ± 120 vs. 163 ± 12 s, $p < 0.001$<br>↑ MCF 64 ± 8 vs. 56 ± 3 mm, $p = 0.001$<br>Contribution of fibrin to clot firmness:<br>↑ CT 104 ± 31 vs. 69 ± 14 s, $p < 0.001$<br>↑ MCF 37 ± 11 vs. 15 ± 4 mm, $p < 0.001$<br>Extrinsic + addition of tPa:<br>↑ CT 68 ± 21 vs. 42 ± 9 s, $p < 0.001$<br>↑ MCF 51 ± 12 vs. 26 ± 9 mm, $p < 0.001$<br>↑ ML 93 ± 15 vs. 92 ± 4%, $p = 0.001$<br>↑ Lysis time 530 ± 327 vs. 211 ± 80 s, $p < 0.001$ | No clinical outcome measures |
| Kruse et al[27]<br>Dec 2020<br>Article | 40 COVID-ICU | After admission<br>*ROTEM*<br>EXTEM, INTEM, FIBTEM, HEPTEM<br>Reference range not provided | *COVID-ICU, median (IQR):*<br>↑ EXTEM MCF 75 (70–78) mm<br>↑ INTEM MCF 74 (69–77) mm<br>↑ FIBTEM MCF 34 (27–39) mm<br>↑ HEPTEM MCF 73 (67–75) mm<br>It is specifically stated as an increase in relation to reference range | *Thrombosis:*<br>23/40 (58%), systematic screening once per week during ICU stay<br>*Thrombosis vs. no thrombosis, median (IQR):*<br>INTEM ML 2 (0–3.0) vs. 6 (2.5–6) %, $p = 0.001$<br>EXTEM ML 3 (0–5) vs. 5 (3.5–8) %, $p = 0.001$ |

**Table 8** (*Continued*)

| Author<br>Publication year and month<br>Publication type | Study characteristics<br>Number of participants<br>Control group | Timing of blood sampling<br>Measurements of hemostasis (reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| Marvi et al[32]<br>Mar 2021<br>Article | 40 COVID-ICU | Within 48 hours of ICU admission<br>*TEG with kaolin/heparinase:*<br>MA (50–70 mm)<br>Alpha angle (53–72°)<br>R-time (5–10 min)<br>Shear elastic modulus (4.5–11 dyne/cm$^2$)<br>Clotting index (−3.0 to +3.0%)<br>Lysis 30 min (0–8%) | *TEG, COVID-ICU, median (IQR) vs. reference range:*<br>→ MA 68.1 (63.6–72.1) mm<br>↑ Alpha angle 72.2 (68.3–75.8)°<br>↓ R-time 3.8 (3.2–4.8) min<br>→ Shear elastic modulus 10.7 (8.8–13.0) dyne/cm$^2$<br>↑ Clotting index 3.4 (3.0–4.1)<br>→ Lysis 30 min 0.6 (0.0–1.4)% | *Thrombosis, in-hospital:*<br>12/40 (30%), 7 PE, 5 DVT<br>*COVID-ICU, thrombosis vs. no thrombosis, median (IQR):*<br>↓ MA 64.4 (62.0–68.7) vs. 70.4 (64.8–73.2) mm, $p = 0.02$<br>↓ Alpha angle 69.0 (66.7–71.2) vs. 74.4 (70.7–77.1°), $p = 0.003$<br>→ R-time 3.9 (3.4–5.5 vs. 3.7 (3.2–4.8) min, $p = 0.44$<br>↓ Shear elastic modulus 9.1 (8.2–11.0) vs. 11.9 (9.2–13.6) dyne/cm$^2$, $p = 0.02$<br>↓ Clotting index 3.1 (2.7–3.4) vs. 3.8 (3.0–4.3), $p = 0.02$<br>→ Lysis 30 min 0.9 (0.2–1.5) vs. 0.5 (0.0–1.2)%, $p = 0.30$ |
| Mitrovic et al[28]<br>Feb 2021<br>Article | 35 COVID<br>Critical (with ARDS)<br>25 COVID<br>Severe<br>34 COVID<br>Moderate<br>Total 94 | Median 9.5 days (range 1–38) from admission<br>*ROTEM*<br>EXTEM<br>CT (38–65 s)<br>CFT (42–93 s)<br>ML (1–12%)<br>MCF (53–68 mm)<br>FIBTEM<br>CT (55–87 s)<br>MCF (9–27 mm) | *COVID critical, median vs. reference range:*<br>EXTEM<br>↑ CT 66 s<br>→ CFT 47 s<br>→ ML 2%<br>→ MCF 68 mm<br>FIBTEM<br>→ CT 85.5 s<br>↑ MCF 32 mm | *Thrombosis:*<br>4/94 (4.3%), 2 PE, 2 DVT<br>*Bleeding:*<br>2/94 (2.1%) |
| Nougier et al[25]<br>Jul 2020<br>Brief report<br>Thrombin generation parameters presented in ►**Table 9** | 48 COVID-ICU<br>30 COVID medical ward<br>30 healthy controls | Within 3 days of admission<br>*ROTEM:*<br>EXTEM with presence of 0.625 µg/mL tPa | *ROTEM + tPa, 19 COVID-ICU vs. 10 healthy, mean ± SD:*<br>↑ MCF 62.3 ± 10 vs. 32 ± 10.4 mm, $p$ = NA<br>↑ [a]Ly30 63 ± 39 vs. 1.8 ± 3.2%, $p$ = NA | *Thrombosis, COVID-ICU:*<br>14/48 (29%), 8 PE, 5 DVT, 1 aortic thrombosis<br>*COVID-ICU, thrombosis vs. no thrombosis, mean ± SD:*<br>[a]Ly30 82 ± 26 vs. 37 ± 35%, $p = 0.0029$ |

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**Table 8** (*Continued*)

| Author<br>Publication year and month<br>Publication type | Study characteristics<br>Number of participants<br>Control group | Timing of blood sampling<br>Measurements of hemostasis (reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| Panigada et al[33]<br>April 2020<br>Brief report | 30 COVID-ICU<br>40 healthy controls | One sample, timing not stated<br>*TEG:*<br>kaolin with heparinase | *COVID-ICU mean (min–max) vs. healthy, mean (lower–upper limit):*<br>R-time 6.3 (3.0–11.9) vs. 10.5 (4.0–8.0)[b] min, 13% < lower limit, 20% > higher limit<br>K-time 1.5 (0.8–2.9) vs. 2.1 (0.0–4.0) min, 0% < lower limit, 0% > higher limit<br>Angle 69.4 (51.1–78.5) vs. 61.7 (47.0–74.0°), 0% < lower limit, 40% > higher limit<br>MA 79.1 (58.0–92.0) vs. 70.8 (54.0–72.0) mm, 0% < lower limit, 7% > higher limit<br>LY30 7.8 (0–54.3) vs. 0 (0–8), 0% < lower limit, 23% > higher limit | No clinical outcome measures |
| Pavoni et al[29]<br>March 2021<br>Article | 20 COVID-ICU<br>20 non-COVID ICU | On admission, day 5 and day 10<br>*ROTEM:*<br>EXTEM<br>CFT (34–159 s)<br>MCF (50–72 mm)<br>ML (3–15%)<br>INTEM<br>CFT (30–110 s)<br>MCF (50–72 mm)<br>ML (3–25%)<br>FIBTEM<br>MCF (9–25 mm) | *Admission, COVID-ICU vs. non-COVID ICU, mean ± SD:*<br>EXTEM<br>↓ CFT 47.4 ± 15.2 vs. 124 ± 31 s, $p < 0.0001$<br>↑MCF 74.3 ± 3.2 vs. 60.4 ± 5.6 mm, $p < 0.0001$<br>→ ML 9.5 ± 5.0 vs. 7.2 ± 3.0%, $p = 0.344$<br>INTEM<br>↓ CFT 41 ± 11.7 vs. 75 ± 23 s, $p = 0.0001$<br>↑ MCF 72.3 ± 6.9 vs. 63.8 ± 5.6 mm, $p = 0.001$<br>→ ML 8.0 ± 6.2 vs.7.0 ± 2.7%, $p = 0.510$<br>↑ FIBTEM MCF 35.1 ± 5.7 vs. 17.4 ± 2.7 mm, $p < 0.0001$ | *ICU mortality, COVID vs. non-COVID:*<br>4/20 (20%) vs. 3/25 (12%)<br>*In-hospital mortality, COVID vs. non-COVID:*<br>1/20 (5%) vs. 0/20 (0%) |
| Spiezia et al[30]<br>April 2020<br>Letter to the editor | 22 COVID-ICU<br>44 healthy | Within 30 minutes of ICU admission<br>*ROTEM:*<br>EXTEM<br>INTEM<br>FIBTEM | *COVID-ICU vs. healthy, mean ± SD:*<br>EXTEM<br>↓ CFT 66 ± 20 vs. 78 ± 26 s, $p = 0.01$<br>↑ MCF 69 ± 6 vs. 64 ± 5 mm, $p = 0.0003$<br>→ ML 1 ± 3 vs. 2 ± 3%, $p = 0.22$<br>INTEM<br>↓ CFT 57 ± 13, 70 ± 18 s, $p = 0.0002$<br>↑ MCF 68 ± 6, vs. 62 ± 7 mm, $p < 0.0001$<br>→ ML 1 ± 3 vs. 2 ± 3%, $p = 0.22$<br>FIBTEM<br>↑ MCF 31 ± 9 vs. 18 ± 6, $p < 0.0001$ | *Thrombosis, in-hospital:*<br>5/22 (23%), 5 DVT |

Abbreviations: AUC, area under the curve; CI, confidence interval; CT, clotting time; CFT, clot formation time; COVID, corona virus disease; DVT, deep venous thrombosis; ICU, Intensive care unit; IQR, interquartile range; MA, maximum amplitude; MCF, maximum clot amplitude; ML, maximum lysis; NA, not available; PE, pulmonary embolism; ROTEM, thromboelastometry; TEG, thromboelastography; tPa, tissue plasminogen activator; SD, standard deviation; VTE, venous thrombus embolism.

[a]Ly30 measures extent of clot breakdown, assessing residual clot firmness.

[b]Possible printing error in the original publication.

**Table 9** Studies included in the systematic review applying thrombin generation assays, $n = 6$

| Author<br>Publication year and month<br>Publication type | Study characteristics<br>Number of participants<br>Control group | Timing of blood sampling<br>Measurements of hemostasis<br>(reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| Bouck et al[34]<br>Jan 2021<br>Article | 20 COVID-ICU<br>26 COVID non-ICU<br>53 sepsis<br>18 healthy | Within 72 hours of ICU admission<br>*Thrombin generation:*<br>PPP<br>Lag time<br>ttP<br>ETP | No difference between ICU and non-ICU COVID patients, therefore presented as one group:<br>*COVID vs. healthy:*<br>No difference in lag time, ttP, ETP<br>Median time to lysis, $p > 0.05$ | |
| Campello et al[35]<br>Feb 2021<br>Article | 30 COVID-ICU<br>59 COVID non-ICU<br>54 healthy | Within 5 days of admission to ICU<br>Thrombin generation, PPP, ± thrombomodulin<br>Lag time, ratio relative to reference plasma<br>ttP, ratio relative to reference plasma<br>ETP | *Thrombin generation, no thrombomodulin, COVID ICU vs. healthy, median (IQR):*<br>↑ Lag time 2.45 (1.92–3.36) vs. 1.11 (1.01–1.24), $p < 0.0001$<br>↑ ttP 1.91 (1.62–2.45) vs. 1.19 (1.10–1.35), $p < 0.0001$<br>→ ETP 1,078 (791–1,272) vs. 1,120 (1,028–1,253) nM*min, $p > 0.05$<br>→ ETP with thrombomodulin 363 (290–443) vs. 493.9 (397.4–620.5) nM*min, $p > 0.05$ | *Thrombosis, in-hospital:*<br>VTE 17/30 (57%)<br>*Bleeding, in-hospital:*<br>5/30 (17%), hereof 2 major bleedings<br>No differences in TG parameters were detected in COVID-19 patients with and without VTE complications |
| Canzano et al[36]<br>Mar 2021<br>Article | 26 COVID ICU<br>20 COVID non-ICU<br>46 healthy | Hospital admission<br>Thrombin generation, ± exogenous tissue factor<br>Peak<br>ETP | *All COVID, no LMWH vs. healthy, without tissue factor, median (IQR):*<br>↓ Lag time 11.3 (10–22.3) vs. 23.3 (18.7–30.2) min, $p =$ NA<br>*COVID-ICU vs. non-ICU, with tissue factor, median (IQR):*<br>↑ Peak 165 (156–169) vs. 40.7 (35–46.4) nmol/L, $p < 0.001$<br>↑ ETP 1,064 (1,040–1,240) vs. 584 (529–638) nM*min, $p < 0.001$ | No clinical outcome measures |
| Chistolini et al[37]<br>July 2020<br>Letter to the editor | 27 COVID ICU | Median 5 days after LMWH treatment commenced<br>*Thrombin generation:*<br>lag time (<4.3 min)<br>ttP (<9.8 min)<br>Peak (<106.2 nM)<br>ETP (<984.12 nM*min) | *COVID-ICU, mean vs. reference range:*<br>↑ Lag time 7.70 min<br>↑ ttP 13.38 min<br>↑ Peak 122.22 nM<br>→ ETP 953.51 nM*min<br>*COVID-ICU, LMWH 100 IE/day vs. LMWH 200 IE/day, mean:*<br>↑ ETP 1,222.52 vs. 705.19 nM*min, $p = 0.01$<br>→ Lag time, ttP and peak, $p > 0.05$ | *Thrombosis:*<br>3/27 (11%), PE 2, myocardial infarction 1—all on low-dose LMWH<br>*Bleeding:*<br>1/27 (4%) |

(*Continued*)

**Table 9** (*Continued*)

| Author<br>Publication year and month<br>Publication type | Study characteristics<br>Number of participants<br>Control group | Timing of blood sampling<br>Measurements of hemostasis<br>(reference range if provided) | Biochemical results | Clinical outcomes |
|---|---|---|---|---|
| de la Morena-Barrio et al[3]<br>April 2021<br>Article | 127 COVID, of these<br>39 COVID-ICU<br>24 non-COVID pneumonia (not ICU) | Baseline sample median 2.0 days following admission (IQR: 1.0–7.0)<br>Thrombin generation, ± thrombomodulin | *Thrombin generation, no thrombomodulin, COVID-ICU vs. COVID non-ICU, mean ± SD:*<br>↑ Lag time 4.4 ± 1.2 vs. 3.9 ± 1.1 min, $p = 0.02$<br>↓ ETP 1,092 ± 255 vs. 1,352 ± 284, $p < 0.001$<br>All other data are provided for the COVID group as a whole | *Thrombosis, incidence (95% CI), COVID-ICU group:*<br>15.2% (5.1–31.9%)<br>*Thrombosis, COVID-ICU vs. COVID non-ICU:*<br>$p = 0.013$<br>*Bleeding, incidence (95% CI), COVID-ICU:*<br>6.1 (0.7–20.2%)<br>*Bleeding events, COVID-ICU vs. COVID non-ICU:*<br>$p = 0.604$<br>Thrombosis vs. no thrombosis:<br>↑ Lag time 5.8 ± 2.3 vs. 3.9 ± 1.0, $p = 0.009$<br>↓ ETP 808 ± 355 vs. 1,312 ± 272, $p = 0.001$<br>Cox regression analysis: low ETP predicted adverse outcome (bleeding, thrombosis, or death) |
| Nougier et al[25]<br>Jul 2020<br>Brief report<br>ROTEM results presented in ►**Table 8** | 48 COVID-ICU<br>30 COVID medical ward<br>30 healthy controls | Within 3 days of admission<br>*Thrombin generation:*<br>PPP-high reagent | *Thrombin generation, COVID-ICU vs. healthy controls (mean ± SD):*<br>→ Peak 312 ± 127 vs. 350 ± 39 nM, $p =$ NA<br>→ ETP 1,682 ± 610 vs. 1,593 ± 206 nM*min, $p =$ NA | *Thrombosis, COVID-ICU group:*<br>14/48 (29%), 8 PE, 5 DVT, 1 aortic thrombosis |

Abbreviations: AUC, area under the curve; CI, confidence interval; COVID, corona virus disease; DVT, deep venous thrombosis; ETP, endogenous thrombin potential; ICU, intensive care unit; IQR, interquartile range; LMWH, low-molecular-weight heparin; NA, not available; PE, pulmonary embolism; PPP, platelet-poor plasma; ttp, time to peak; SD, standard deviation; VTE, venous thrombus embolism.

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tPa.[7,25] Nougier et al found higher residual clot firmness (Ly30) in ICU COVID-19 patients than in healthy controls,[25] while Heinz et al found significantly longer lysis time in ICU COVID-19 patients than in healthy controls.[7]

Five studies used the commercially available standard ROTEM or TEG assays to evaluate fibrinolysis. Maximum lysis or lysis at 30 minutes was within the reference range,[9,28,32] comparable to healthy controls[30] or non-COVID-19 ICU patients.[29]

### Coagulation Parameters and Clinical Outcome

Six studies related their biochemical findings to a clinical outcome, this being mortality, thrombosis, or bleeding.[3,25–27,31,32] None of the three studies investigating primary hemostasis reported outcome data. In the study by Bocci et al, there was no difference in any TEG6s parameter between patients who were dead ($n = 17$) or alive patients ($n = 17$) after 28 days.[31] In a French study by Nougier et al, in a subset of the patients included in the study, the ROTEM assay parameter Ly30 (residual clot firmness at 30 minutes) was higher among those with thrombosis (mean $82 \pm 26\%$) than in those without ($37 \pm 35\%$).[25] Likewise, Kruse et al found lower EXTEM and INTEM maximum lysis in 23 patients developing thrombosis (EXTEM: median 3%, IQR: 0–5 and INTEM: 2%, IQR: 0–3) than in 17 patients without thrombosis (EXTEM: median 5%, IQR: 3.5–8 and INTEM: 6%, IQR: 2.5–6).[27] In contrast, Marvi et al found comparable percent lysis at 30 minutes between patients developing venous thromboembolism (0.9%, IQR: 0.2–1.5) and patients not developing thrombosis (0.5%, IQR: 0.0–1.3). However, patients with thrombosis had slightly lower maximum amplitude and α-angle in comparison to patients not developing thrombosis: maximum amplitude: 64.4 (62.0–68.7) versus 70.4 (64.8–73.2) mm and α-angle: 69.0 (66.7–71.2) versus 74.4 (70.7–77.1).[32] In the study by de la Morena-Barrio et al, a low ETP was associated with a composite outcome of death, thrombosis, and bleeding (unadjusted hazard ratio = 0.87, 95% confidence interval: 0.77–0.99).[3]

## Discussion

The literature search for the systematic review revealed >3,000 publications containing data on coagulation in COVID-19 patients. A remarkably large proportion were reviews and only 18 studies collected data prospectively, included more than 20 ICU COVID-19 patients, and applied dynamic coagulation assays. The relation between the biochemical findings and development of thrombosis or bleeding was only sparsely investigated and showed conflicting results. An association between coagulation parameters and development of thrombosis could not be discerned. This sparse evidence gave rise to the present comprehensive investigation of COVID-19 ICU-patients employing a wide range of dynamic coagulation and fibrinolysis assays.

The main finding of the current study was an increased in vivo thrombin generation in ICU COVID-19 patients. Patients developing thrombosis had higher levels of TAT complex levels and F1 + 2 concentrations on admission compared with ICU patients not developing thrombosis. In contrast, the hypercoagulation presented in the ROTEM assay was not associated with development of thrombosis. Fibrinolysis was reduced during the first week of the ICU stay, but levels of fibrinolysis plasma markers or measurements from the dynamic clot lysis assay did not differ between patients developing thrombosis versus those without. Hence, at this stage of disease, the primary determinant for thrombosis seems to be increased thrombin generation in vivo occurring despite LMWH prophylaxis. Platelet aggregation was slightly reduced on admission to ICU, but aggregation did not differ between patients experiencing bleeding and patients not experiencing this complication.

The cohort study confirms previous studies identified by the systematic review that indicated preserved or slightly reduced platelet aggregation.[7–9] In addition, the present study demonstrated no association between changes in platelet aggregation and thromboembolic risk and the slightly reduced platelet aggregation did not seem to influence the risk of bleeding. Others have described increased levels of platelet-associated cytokines as well as release of α- and dense-granule contents in both ICU and non-ICU COVID-19 patients,[38,39] but these findings were not related to the development of thrombosis.[38] The level of von Willebrand factor was also found to be increased[39,40] and with increased binding capacity,[40] but still platelet aggregation was reduced upon stimulation.[40] It is speculated that platelet exhaustion due to increased platelet activity in the early course of COVID-19 is a possible mechanism behind this consistent finding of reduced platelet aggregation later in the course of disease.[40]

Whether the risk of thrombosis or severity of the disease can be modulated by acetylsalicylic acid (ASA) has been investigated in two ways, but only in non-ICU populations. First, a meta-analysis by Srivastava and Kumar investigated if the continuation of ASA upon hospitalization due to COVID-19 was beneficial.[41] The meta-analysis included 10 studies of which nine were retrospective and with great heterogeneity. The meta-analysis showed reduced odds ratio for death in patients receiving ASA, an effect driven by one study and disappearing after exclusion of outliers.[41] Second, the RECOVERY group investigated the commencement of treatment with ASA upon hospitalization due to COVID-19 in a randomized controlled trial.[42] The study included 14,892 patients allocated to receive ASA or usual treatment. ASA use was associated with an absolute reduction in thrombotic events of 0.6% but also an absolute increase in major bleeding events of 0.6% and 28-day mortality was not affected by ASA treatment.[42] Hence, the use of platelet inhibitors may not provide clinical benefit in COVID-19 requiring ICU admission.

Our systematic review and cohort study confirmed prolonged clot initiation, normal or increased clot propagation, as well as increased clot firmness among ICU patients with COVID-19. However, discrepancies exist, as the studies by Panigada et al and Marvi et al[32,33] revealed shortened clotting time, and presented purely hypercoagulable patterns. Both of the latter studies were performed employing

kaolin and heparinase, while we performed our analysis without heparinase added. The increased clot firmness was presumably explained by the high level of fibrinogen. Ex vivo thrombin generation measured by the thrombin generation assay was in the cohort study equivalent to the healthy controls. This is in accordance with the findings in the systematic review. Nougier et al argue that normal thrombin generation in ICU COVID-19 patients is in fact hypercoagulation as the patients receive heparin. We measured anti-factor Xa and found a correlation between this and the ETP. However, there are several caveats in interpreting on anti-factor Xa levels in relation to thrombin generation, as, depending on the anti-factor Xa assay used, heparin amounts may be overestimated, particularly in case of low concentrations.[43] The changes in ROTEM/TEG parameters did not seem to influence the risk of thrombosis in the cohort study or in the majority of studies in the systematic review.[26,31] Only in the study by Marvi et al, the maximum amplitude was lower in patients developing thrombosis than in patients not developing thrombosis, which is difficult to explain. Neither did the ROTEM/TEG parameters in the present cohort study differ between patients bleeding and not bleeding, while the studies included in the systematic review did not provide data on bleeding. Hence, the present cohort study and review does not support ROTEM/TEG as monitoring tools for development of thrombosis. Data on the association between ROTEM/TEG parameters and clinical bleeding are scarce.

The ex vivo thrombin generation measurements illustrate the thrombin generation capacity, while the in vivo measurements reflect pathological activation.[44] The ex vivo thrombin generation parameters were not related to development of thrombosis, neither in the cohort study, nor in the systematic review. De la Morena-Barrio et al related a low ETP to adverse outcomes, but applied a composite outcome of thrombosis, bleeding, and death, which may be challenging to interpret. None of the studies in the systematic review, relating the coagulation parameters to outcome, measured in vivo thrombin generation parameters. Others have measured TAT complex levels and $F1+2$ concentrations in ICU COVID-19 patients.[45–47] Blasi et al[45] and Hekimian et al[47] found increased levels of both proteins, but none of the studies related their findings to clinical outcome. In the present cohort study an increased in vivo thrombin generation early in the ICU admission was related to development of thrombosis. Hence, current evidence supports increased secondary hemostasis in ICU patients with COVID-19, possibly related to the formation of thrombosis. This supports the use of heparin as prophylaxis, and in the present cohort study, intermediate dosing of LMWH was well tolerated.

The role of fibrinolysis in COVID-19-associated coagulopathy is under debate.[48] Few studies employing dynamic clot lysis assays have been published[45,49] and none fulfilled the inclusion criteria of this systematic review. Only two of the included studies investigated fibrinolysis in dynamic assays by addition of tPa.[7,25] Both found increased lysis resistance in ICU COVID-19 patients in comparison to healthy individuals. We expanded this finding, as lysis time was prolonged in our ICU COVID-19 cohort in comparison to healthy controls. Maximum lysis is interpreted on in several studies included in the systematic review,[9,27,29,30] but we want to stress that caution is needed in the interpretation of maximum lysis of standard ROTEM assays due to the limitations of the assay.[43]

Both the systematic review and the present cohort study support that ICU COVID-19 patients are in a hypofibrinolytic state. However, the few studies that related their findings to outcome, including the present cohort study, did not find a link between hypofibrinolysis and development of thrombosis. However, the use of tranexamic acid in the treatment of the bleeding COVID-19 patient is not encouraged, as it may in theory promote thrombosis.

The strengths of the present cohort study are the application of a wide range of dynamic assays performed on prospectively collected blood samples and clinical information. Further, the cohort was very well characterized and a comprehensive baseline description is provided. Patients were followed for a week with blood samples and none was lost to 30-day follow-up. The present cohort study suffers from a limitation in sample size. This issue was aggravated by particularly the clot lysis assay being sensitive to heparin treatment. This resulted in missing data, possibly leading to a missed association between hypofibrinolysis and development of thrombosis. In addition, platelet aggregation was investigated by a method not employing shear stress, and used a limited number of agonists; moreover, platelet activation as well as secretion was not investigated. Likewise, endothelial secretions, e.g., von Willebrand factor, were not measured.

The strength of the systematic review was a broad search string including all studies on COVID-19 patients, which evaluated coagulation parameters. Thus, the risk of overlooking potential eligible studies was low. However, only 18 studies were eligible for inclusion and very few of those related their findings to clinical outcome. Studies were mainly excluded due to small sample size, retrospective design, and non-ICU cohort. Further, the included studies were still small in sample size and mostly with cross-sectional design. The dynamic assays applied differed between studies, not all assays were standardized and some were modifications of standard assays.

## Conclusion

The present hybrid manuscript, presenting a systematic review and a cohort study employing a wide range of investigations, reveals that a clear association between specific changes in coagulation seen in ICU COVID-19 patients and development of thrombosis or bleeding cannot be discerned. However, our cohort study did reveal a link between increased in vivo thrombin generation and development of thrombosis, which supports the use of heparin in ICU COVID-19 patients.

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Conflict of Interest
None declared.

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