

Prognostic value of thrombin generation parameters in hospitalized COVID-19 patients

María Eugenia de la Morena-Barrio

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

Carlos Bravo-Pérez

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

Antonia Miñano

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

Belén de la Morena-Barrio

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

María Piedad Fernandez-Perez

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

Enrique Bernal

Servicio de Enfermedades Infecciosas, Hospital Reina Sofía

José Miguel Gómez-Verdu

Servicio de Medicina Interna, Hospital Universitario Morales Meseguer

María Teresa Herranz

Servicio de Medicina Interna, Hospital Universitario Morales Meseguer

Vicente Vicente

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

Javier Corral (✉ javiercorraldelacalle@gmail.com)

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

María Luisa Lozano

Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia

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Abstract

Background. SARS-CoV-2 infection increases the risk of thrombosis by different mechanisms not fully characterized. Although still debated, an increase in D-dimer has been proposed as a first-line hemostasis test associated with thromboembolic risk and unfavorable prognosis.

Objective. We aim to systematically and comprehensively evaluate the association between thrombin generation parameters and the inflammatory and hypercoagulable state, as well as their prognostic value in COVID-19 patients

Methods. A total of 127 hospitalized patients with confirmed COVID-19, 24 hospitalized patients with SARS-CoV-2-negative pneumonia and 12 healthy subjects were included. Clinical characteristics, thrombin generation triggered by tissue factor with and without soluble thrombomodulin, and also by silica, as well as other biochemical parameters were assessed.

Results. Despite the frequent use of heparin, COVID-19 patients had similar thrombin generation than healthy controls. In COVID-19 patients, the thrombin generation lag-time positively correlated with markers of cell lysis (LDH), inflammation (CRP, IL-6) and coagulation (D-dimer), while the endogenous thrombin potential (ETP) inversely correlated with D-dimer and LDH, and positively correlated with fibrinogen levels. Patients with more prolonged lag-time and decreased ETP presented with increased ISTH-DIC scores, and had more severe disease (vascular events and death). The ROC curve and Kaplan Meier estimate indicated that the D-dimer/ETP ratio was associated with in-hospital mortality (HR 2.5; $p=0.006$), and with the occurrence of major adverse events (composite end-point of vascular events and death) (HR 2.38; $p=0.004$).

Conclusions. The thrombin generation ETP and lag-time variables correlate with thromboinflammatory markers, and the D-dimer/ETP ratio can predict major adverse events in COVID-19.

Introduction

The clinical manifestations of SARS-CoV-2 infection are highly heterogeneous, from asymptomatic disease to devastating outcomes. The most characteristic complication is acute respiratory distress syndrome (ARDS), but thrombotic complications are also frequent, especially in patients admitted to the intensive care unit (ICU).¹⁻⁹ Thrombosis may be justified by excessive inflammation, hypoxia, immobilization, and microvascular injury.¹⁰⁻¹⁵ Despite the strides that have been made in the last months in understanding the mechanisms underlying COVID-19 associated coagulopathy, efforts are still necessary to decipher its complexity. The hemostatic profile of COVID-19 infection is different from sepsis coagulopathy or disseminated intravascular coagulation (DIC).¹⁶⁻²² Endothelial dysfunction and platelet activation may support thrombin generation. The activation of the complement system pathways may contribute to the production of cytokines and chemokines, and also phenomena of hypofibrinolysis could ultimately lead to immunothrombosis.²³ With limited solid data available, it is crucial to identify biomarkers that may help predict poor outcomes, including thrombotic events. In addition to D-dimer, most attempts have been mainly focused on the detection of new thromboinflammatory biomarkers, but studies analyzing global hemostatic methods are rare. In this disease, the correlation of thrombin generation parameters with biomarkers of immunothrombosis or with clinical outcomes has not been evaluated, which could provide some additional useful information not only in our understanding of the physiopathology of the disease, but could also help for stratification and managed care of COVID-19. The aim of this study was to systematically investigate the potential association of thrombin generation with other biochemical variables, and to assess the prognostic value of this assay in patients with COVID-19.

Materials And Methods

Patients

Consecutive patients with COVID-19 requiring hospital admission at Morales Meseguer and Reina Sofia University Hospitals (Murcia, Spain) from March to April 2020 were included in this cross-sectional study. In all patients, SARS-CoV-2 infection was assessed by quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) using the diagnostic criteria defined in the World Health Organization (WHO) interim guidance²⁴. Exclusion criteria included chronic oral anticoagulation at

the time of blood sampling, patients younger than 18 years of age, or those with missing data or who were transferred from other designated hospitals. A total of 127 hospitalized patients were included in the analysis, with a final follow-up date July 14, 2020.

As comparative groups, patients requiring admission at the same period with SARS-CoV-2-negative pneumonia were also enrolled. In these cases, COVID-19 diagnosis was ruled out by repetitively negative SARS-CoV-2 qRT-PCR and serologic testing. Samples from 12 healthy individuals and a pool of plasma from 100 healthy subjects were also analyzed for thrombin generation.

All included subjects gave their informed consent to enter the study, which was approved by the Ethics Committee of Morales Meseguer Hospital, and performed in accordance with the 1964 Declaration of Helsinki and their later amendments.

Sample collection

Samples were collected during hospitalization in vacuum tubes containing 0.109 M sodium citrate. Platelet-poor plasma (PPP) was obtained within 4 hours after extraction by two cycles of centrifugation (2000g x15 min) at 20°C and then stored at -80°C.

Data collection

Three investigators (MTH, JMGV, and EB) independently extracted data including demographics, comorbidities, laboratory studies and outcomes, using a standardized data collection form for analysis. Such data were checked independently by three investigators (CBP, MPFP and MEMB) to verify accuracy. The clinical information comprised patient demographics, medical comorbidities, prior thrombotic history, chronic anticoagulation, length of hospital stay, CURB-65 score at admission, development of ARDS, ICU admission, sequential organ failure assessment (SOFA) score, vascular (thrombotic/ hemorrhagic) events, antithrombotic therapy while hospitalization, and death.

Venous and arterial thrombotic events were confirmed by radiologic methods (Doppler-Ultrasonography [US] and Computed Tomography [CT]). Bleeding was graded according to World Health Organization (WHO) scale. Grade ≥ 3 bleeding episodes, requiring transfusion, were confirmed by angiographic procedures. Routine laboratory parameters were recorded at admission. Their peak levels during follow-up were also documented. These included lactate dehydrogenase (LDH), C reactive protein (CRP), and interleukin 6 (IL-6). Complete blood cell counts and basic coagulation tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen and D-dimer) were also obtained. COVID-19 associated coagulopathy was graded according to DIC score of the International Society on Thrombosis and Haemostasis (ISTH).²⁵ Prescribed antithrombotic therapy with low molecular weight heparin (LMWH), specifically enoxaparin, was classified as standard prophylaxis (40 mg QD), high-risk prophylaxis (1 mg/Kg QD), and therapeutic regimens (1 mg/Kg BD).

Thrombin generation assay

The thrombin generation assay (TGA) was performed using the calibrated automated thrombogram (CAT) method.²⁶ All samples were evaluated in the same equipment during three working days using the same reagent batch under the following conditions: samples were thawed at 37°C and mixed with two different activators: PPP reagent® (5 pM tissue factor [TF]; 4 μ M phospholipids) (Stago Diagnostics) or silica (dilution 1/16 from Synthasyl reagent; Werfen), in a 96-well plate (Immulon, 2HB clear U-bottom; ThermoFisher Scientific). All samples were run in duplicate. Coagulation was triggered with calcium chloride in buffer containing the fluorogenic substrate (FluCa-kit reagent®, Stago Diagnostics). For each individual plasma sample, we used a thrombin calibrator (Stago Diagnostics) to correct for differences in sample color, inner filter fluorescence, and substrate consumption. Fluorescence was recorded for 60 min in a Fluoroskan Ascent microplate fluorimeter (Thermolab Systems, Helsinki, Finland), and the data were then analyzed using Thrombinoscope™ software (version 5.0.0.742, Stago Diagnostics). The endogenous thrombin potential (ETP), the thrombin peak, the time to the thrombin peak, the velocity index and the lag-time were recorded for each assay. To assess the impact of the protein C anticoagulant pathway on the TGA, experiments activated with PPP reagent were also conducted in the presence of recombinant human soluble thrombomodulin (sTM) (American Diagnostica) at a final concentration of 7.5 nM. An index “R” described by Perrin et al,²⁷ was calculated for the TGA parameters, i.e. the value in the presence of sTM divided by the value in the absence of sTM; the closer the ratio is to 1, the weaker the response to the protein C system.

Statistical analysis

Descriptive analysis of qualitative variables included percentages. Pearson's Chi-Squared test and Fisher's Exact test were used for comparison of proportions or ordinal variables. Kolmogorov-Smirnov and Shapiro-Wilk tests were used for testing normality of continuous variables. Normally distributed continuous variables were presented as means \pm standard deviations (SD), whereas non-normally distributed variables were presented as median and interquartile ranges (IQR). Student's-T (parametric) or Mann-Whitney-U tests were used for comparison of two means and analysis of variance (ANOVA, parametric) or Kruskal-Wallis (non-parametric) tests were used for comparison of more than two means. Apart from p values, 95% Confidence Intervals (95%CI) were also calculated.

Association between thrombin generation and other continuous laboratory variables was assessed by Pearson's (parametric) or Spearman's Rho (non-parametric) correlation tests.

The predictive impact of thrombin generation and other laboratory parameters on the incidence of clinical events (hemorrhage, thrombosis, ARDS, ICU admission and death) was assessed by Cox regression analysis. To explore an optimal cut-off value, a Receiver-Operating-Characteristic (ROC) curve analysis was performed, and survival analysis was assessed using the Kaplan-Meier method and compared using log-rank tests.

Logarithmic and square root transformations of continuous variables were performed to make the data more closely meet the assumptions of statistical procedures to be applied, or to improve their interpretability.

Statistical analysis was performed with the use of Excel® (Microsoft), GraphPad Prism® (GraphPad Software), IBM SPSS Statistics 21® (IBM SPSS Software) and STATA v.14® (StataCorp LLC).

Results

Patients' characteristics

The basic characteristics for all participants are shown in Table 1. COVID-19 patients were older than those with SARS-CoV-2 negative pneumonia (median age 60 years [IQR, 47.5-72.3 years] vs. 37 years [IQR 29.5-31.7 years]), respectively; $p < 0.001$). The median time from admission to discharge was 10 days in COVID-19 patients, and 2 days in SARS-CoV-2 negative pneumonia patients ($p = 0.002$). COVID-19 patients had significantly higher rates of hypertension, diabetes, dyslipidemia, and chronic renal failure, whereas SARS-CoV-2 pneumonia patients had higher rates of history of smoking ($p \leq 0.05$). The results also showed that 30.7%, and 26.0% of COVID-19 patients developed ARDS and required ICU admission, respectively, and that 7.9% of the COVID-19 cohort had fatal outcomes. Patients in the SARS-CoV-2-negative pneumonia group had much milder clinical manifestations.

Antithrombotic prophylaxis, thrombosis, and bleeding events in COVID-19 patients

Antithrombotic therapies, thrombotic events, and bleeding complications in patients with COVID-19 and with SARS-CoV-2 negative pneumonia are summarized in Table 2.

In the COVID-19 cohort, 7 patients experienced 8 thrombotic events. These included 5 venous episodes (4 pulmonary embolism [PE], 1 extensive superficial venous thrombosis), and 3 ischemic episodes (1 stroke, 1 acute coronary syndrome, and 1 acute peripheral arterial ischemia). Thus, the incidence of thrombosis in COVID-19 patients in our cohort was 5.5 % (95%CI: 2.2-11.0 %); 3.9 % (95%CI: 1.3-8.9 %) for radiographically-confirmed venous thromboembolism and 2.4 % (95%CI: 0.5-6.7 %) for arterial thrombosis. Five out of these 7 patients (71.4%) had critically-ill COVID-19 and required ICU admission, rendering an incidence of thrombosis of 15.2% (95%CI: 5.1-31.9%) in ICU patients. One of the patients developed two vascular events, a symptomatic PE followed 7 days later by acute myocardial infarction despite full-dose anticoagulation with heparin. Of note, thrombotic complications seemed to occur despite antithrombotic measures, since 85.8% of patients with thrombosis received pharmacological thromboprophylaxis, and 28.6% of them intermediate/therapeutic dose heparin.

Five bleeding events occurred in 5 COVID-19 patients: 2 retroperitoneal hematomas, 1 rectus sheath hematoma (these 3 episodes had a WHO Severity Grade ≥ 3), 1 upper gastrointestinal bleeding and 1 episode of hemoptysis. Therefore, the rate of bleeding in

COVID-19 patients from our cohort was 3.8 % (95%CI: 1.2–8.6 %) and 2.3 % (95%CI: 0.5-6.5 %) for WHO grade ≥ 3 bleeding. Two out of these 5 patients (40.0%) had critically-ill COVID-19 and required ICU admission, resulting in an incidence of bleeding of 6.1 % (95%CI: 0.7–20.2%) in ICU patients. All cases received thromboprophylaxis with heparin. Notably, at the moment of bleeding, the 3 patients with Grade ≥ 3 internal hemorrhages were on intermediate/therapeutic-dose heparin.

No vascular events were recorded in patients with SARS-CoV-2-negative pneumonia.

Laboratory data

Laboratory parameters were analyzed and the results showed that the levels of LDH and D-dimer were higher in COVID-19 patients than those in the SARS-CoV-2 negative pneumonia group. In terms of blood counts and other coagulation indexes, the COVID-19 group showed significantly lower trough platelet counts and PT compared with the SARS-CoV-2 negative pneumonia group. Thus, in the COVID-19 cohort, 11.0% of patients had thrombocytopenia ($<150 \times 10^9/L$), 30.7% had prolonged PT ($<75\%$) and/or aPTT ratio (>1.30) and 0.8% fibrinogen consumption ($<100 \text{ mg/dL}$). Meanwhile, baseline and peak ISTH-DIC score values were also significantly higher in COVID-19 patients than those in the SARS-CoV-2 negative pneumonia group (Table 3).

Notably, at admission, no patient met criteria of overt DIC (score ≥ 5), but in 51.9% of them, the profile was consistent with low-grade coagulopathy (scores 1-4). During follow-up, 64.7 % cases showed low-grade coagulopathy, and 3.8 % eventually met criteria of overt DIC. Both baseline and peak ISTH-DIC scores were higher in patients suffering from bleeding than those who did not (median baseline ISTH-DIC score: 2 [IQR: 1.25-2.75] for bleeders and 0 [IQR: 0–1] for non-bleeders, $p= 0.005$; median peak ISTH-DIC score: 3.5 [IQR: 1.5–4] for bleeders and 1 [IQR: 0-2] for non-bleeders, $p= 0.016$).

Thrombin generation.

The results of the thrombin generation parameters in COVID-19 and SARS-CoV-2 negative pneumonia from the study sample and reference values (healthy subjects) are shown in Table 4. Despite 78.9% of COVID-19 patients (N= 105) were undergoing LMWH treatment, when thrombin generation was triggered either by TF or silica, COVID-19 patients had similar lag-time and ETP but higher peak than healthy controls. At 5 pM TF, SARS-CoV-2 negative pneumonia patients presented with a shorter lag-time, higher ETP and higher peak height compared to COVID-19 samples, most likely due to the lower proportion of patients receiving LMWH than COVID-19 patients (Table 4 and Figure S1). In the study sample, when sTM was added, as an agent that converts thrombin into an anticoagulant capable of activating protein C, it could not significantly reduce the ETP in patients with pneumonia (COVID-19 and SARS-CoV-2 negative), compared with the effect expected and observed in samples from healthy subjects (Table 4).

Correlation of thrombin generation parameters with analytical data in COVID-19 patients

As shown in Table 5, in COVID-19 patients when thromboinflammatory markers were dichotomized according to median values, higher LDH, CRP, IL-6, and D-dimer values were associated with longer lag-time triggered by TF. Additionally, increased LDH and fibrinogen levels, but lower D-dimer values were associated with lower ETP at 5 pM TF (Table 5). Results from multiple linear regression analysis for TGA parameters at 5 pM TF, demonstrated associations with markers of cell lysis (LDH), inflammation (CRP, IL-6), and coagulation (fibrinogen and D-dimer). The lag-time was positively associated with all the above biomarkers except for fibrinogen. Otherwise, ETP was inversely associated with LDH and D-dimer, and positively related to fibrinogen levels, as shown in Table 5 and supplementary figure S2.

Thrombin generation and clinical severity

We analyzed whether in COVID-19 patients, TGA measured in PPP with 5 pM TF associated with clinical complications. Outcomes of interest included CURB-65 score (a scoring system for assessing the severity of pneumonia), ICU admission, ARDS, death, thrombosis, and the composite for adverse events that includes ARDS, thrombosis, hemorrhage or death. Of the various outcomes considered, all of them showed a positive association with ETP, and also (except for death) with lag-time (Table 6).

As an additional main outcome, and according to the criteria defined by the ISTH to be applied to patients with a critical illness known to precipitate DIC, the ISTH-DIC score was evaluated, and the number of clinically relevant episodes (ADRS, thrombosis, bleeding or death) were also scored during follow-up. TGA showed lower ETP levels but longer lag-times in patients who experienced more clinical events and had higher ISTH-DIC scores (Figure 1)(Table 6).

Predictive value of thrombin generation assay in COVID-19

Cox regression analysis was performed to evaluate the predictive value for TGA parameters of adverse clinical events of in patients hospitalized with COVID-19 infection. For this analysis, individuals experiencing adverse events before three days following sample collection were excluded (N= 6). Cox regression analysis confirmed that despite the low number of events, ETP and D-dimer significantly predicted adverse outcomes (thrombosis, bleeding or death), being the ratio D-dimer/ETP the best predictor (Figure 2).

The ROC curve also showed good specificity and sensitivity for the D-dimer/ETP ratio and adverse events in COVID-19 patients (AUC: 0.833, 95%CI: 0.719–0.948, $p < 0.001$) (Figure 3A) and using the optimal cut-off value of 1.63 obtained from the ROC curve, the Kaplan-Meier estimation also reflected that the D-dimer/ETP ratio was significantly related to adverse events ($p = 0.016$, HR: 0.153) (Figure 3B).

Discussion

It is now accepted that coagulopathy and DIC associate with a poor prognosis in patients with COVID-19, although it is as yet unknown if this is directly related to SARS-CoV-2 damage, or more likely due to the effects of hypoxia and sepsis.²⁸ The high incidence of thrombosis in COVID-19 strongly supports a significant disturbance of the hemostatic system in these patients, that however is only mainly sustained by the increase of D-dimer.²⁹ Thus, further clues on the status of the hemostatic system in patients with COVID-19 are required. Moreover, as subsequent waves of infections appear, we need new and stronger prognostic biomarkers to guide clinical decision making in order to triage which patients can be discharged or may need admission, or to identify high-risk cases that might need admission in ICU. At the present time, current prophylaxis seems to be inefficient in some patients, which could however associate with an increased bleeding risk,³⁰ so that more individualized approaches to guide thrombosis prevention could help in the tailored management of patients.³¹ Routine coagulation tests do not seem useful to discriminate severe cases of COVID-19, as recently suggested.²¹ Clotting times (PT and aPTT) do not enable the correct appreciation of coagulation profile in patients with coagulopathy because they take into account only extreme reductions in the levels of pro-coagulant factors and are not sensitive to the concomitant reduction of inhibitors. In this framework, global tests of hemostasis, which are closer to the *in vivo* coagulation conditions and reflect the capacity of response of the hemostatic system to a triggering factor, might be of great value.³² However, to our knowledge only three studies have used other global hemostatic tests apart from coagulation times to evaluate the hemostatic system in patients with COVID-19. Such studies, two using thromboelastography and the third also using a TGA, included a relatively limited number of patients (22, 24 and 78, respectively), and the first was restricted to patients admitted to ICU.^{33–35}

Here, we used a CAT TGA to analyze the response of the hemostatic system to TF both in the absence and presence of sTM (therefore evaluating the extrinsic pathway and the protein C system, respectively), but also by activating the contact pathway with silica. Additionally, we studied the association of TGA parameters with analytical and clinical data, which may help to understand the relationship of changes in the hemostatic system with thromboinflammatory biomarkers and with clinical outcomes. For that, the assay was done in a single equipment under strict conditions aiming to reduce any variability, and with appropriate controls, which also included patients with SARS-CoV-2-negative pneumonia.

Results confirm previous findings showing a hypercoagulable state in COVID-19 patients, as they have similar thrombin generation than healthy controls despite thromboprophylaxis.^{33–35} Results also reflect the impairment of the protein C pathway, as the addition of sTM, both in COVID-19, and in patients with SARS-CoV-2-negative pneumonia, resulted in minor reduction in the generation of thrombin compared to controls. These results are in line with those suggesting that infections may trigger the release of TM from the damaged endothelium.³⁶ This reduction of the antithrombotic capacity at the surface of the

endothelium,^{37,38} may contribute to fibrin deposition, particularly at the lung³⁹. Further studies of sTM in these patients might be useful to validate this hypothesis.

Our study also shows a good correlation of TGA parameters with markers of cell lysis (LDH), inflammation (IL-6; CRP) and coagulation (fibrinogen and D-dimer). Interestingly, patients with higher inflammation and cell lysis have prolonged lag-time and reduced ETP, probably reflecting a consumption of coagulation factors of the hemostatic system. In this framework, the association of lag-time and ETP with D-dimer is also remarkable. The fact that patients with higher D-dimer had prolonged lag-time and reduced ETP supports a coagulopathy in severe COVID-19 patients. This finding was also reinforced by the excellent association of ETP with the ISTH-DIC score. Thus, our results would indicate both, a hypercoagulable state, and a low-grade coagulopathy in COVID-19 patients.

Finally, the association of TGA parameters with clinical outcomes is also noteworthy. In contrast to that expected for a hypercoagulable state, lower ETP was associated to worse prognosis according to at-admission CURB-65 score and the development of adverse events (ARDS, vascular events, or death). Those patients with reduced capacity to generate thrombin have poor prognosis, suggesting that the severity of the disease is related to a low capacity of response of the hemostatic system, probably because of the associated consumptive coagulopathy. In accordance, our study confirms the prognostic value of D-dimer levels on mortality, which has been debated,⁴⁰. Additionally, these data also suggest that TGA parameters, especially the D-dimer/ETP ratio might have better prediction ability for vascular events and death. The main limitation of our study is the lack of levels of thrombin activity before initiation of heparin, which would allow more complete characterization of the effect of the anticoagulant on thrombin generation over time, and also the possibility that medical records were incomplete or missing data. A strength of the approach is the use of a representative patient sample that includes patients managed in two distinct institutions. Thus, our results suggest that thrombin generation could have a promising prognostic value in COVID-19 patients, but more extensive testing is warranted to validate these findings before being incorporated into clinical practice.

Overall, our study supports the results of previous reports showing multiple pathogenic pathways in COVID-19 patients. The severe “storm” of proinflammatory cytokines, combined with cell lysis, particularly at the endothelium, constitute insults leading to a significant hypercoagulable state that, despite antithrombotic prophylaxis, cause a consumptive and diffuse coagulopathy reflected by the increase of D-dimer, independently of the hypofibrinolysis that is also present in these patients.⁴¹ Thus, patients with lower capacity of thrombin generation and higher D-dimer levels would have poor prognosis (Figure 4).

Declarations

Author contributions

ME M-B, C B-P, AM and B M-B recruited the samples and performed experimental analysis. ME M-B, C B-P and MP F-P statistically analyzed all data. E B, JM G-V, MT H recruited patients and clinical outcomes and designed the research. V V, J C, ML L designed the research, wrote the paper. All authors read and approved the final manuscript

Additional Information

Ethics approval and consent to participate

All included subjects gave their informed consent to enter the study, which was approved by the Ethics Committee of Morales Meseguer Hospital, and performed in accordance with the 1964 Declaration of Helsinki and their later amendments.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1. Demographic and clinical characteristics of patients with COVID-19 and SARS-CoV-2 negative pneumonia.

	COVID-19 (N = 127)	SARS-CoV-2-negative Pneumonia (N = 24)	p
Demographics			
Sex (females)	43.3 % (N=55)	54.2% (N=13)	0.327
Age (years), median (IQR)	60 (47.5 - 72.3)	37 (29.5 - 31.8)	<0.001
Comorbidities			
Hypertension	47.0 % (N=61)	12.5 % (N=3)	0.002
Diabetes mellitus	18.1 % (N=23)	0 %	0.016
Dyslipidemia	36.2 % (N=46)	0 %	<0.001
Cardiovascular disease	14.2 % (N=18)	4.2 % (N=1)	0.135
Chronic lung disease	1.6 % (N=2)	8.3 % (N=2)	0.089
Smoking history	17.3 % (N=22)	37.5 % (N=9)	0.049
Chronic renal disease	18.9 % (N=24)	0 %	0.020
Cancer	4.7 % (N=6)	0 %	0.590
Immunosuppression	4.7% (N=6)	0 %	0.590
At-admission severity			
CURB-65 ≥ 2, % (95%CI)	30.7 % (22.8-39.5 %) (N=39)	0 % (0 - 14.2%)	0.002
Length of stay, median (IQR)	10 days (5-19 days)	2 days (1 - 3.8 days)	<0.001
Evolution to critically-ill disease	30.7 % (22.8-39.5 %) (N=33)	0 % (0 - 14.2%)	0.002
	26.0 % (18.6-34.5 %) (N=39)	0 % (0 - 14.2%)	0.005
ARDS, % (95%CI)	0 (0 - 2)	0 (0 - 1)	0.020
ICU admission, % (95%CI)	4 (0 - 7.5)	-	-
SOFA score, median (IQR)			
SOFA of ICU patients, median (IQR)			
Deaths, % (95%CI)	Overall: 7.9 % (3.8-14.0%) (N=10)		0.004*
	ICU Non-ICU	0 %	
	18.2 % (7.0-35.5%) (N=6) 4.3 % (1.3-4.2%) (N=4)	(0 - 14.2%)	
	p = 0.019		

* p value of Chi-Squared test for 3 categories (COVID-19 ICU and non-ICU and SARS-CoV-2-negative Pneumonia).

ARDS: acute respiratory distress syndrome. ICU: intensive care unit. IQR: interquartile range. SOFA score: Sequential organ failure assessment score

Table 2. Antithrombotic therapies and vascular events in COVID-19 patients and with SARS-CoV-2 negative pneumonia.

	COVID-19 (N= 127)	SARS-CoV-2-negative Pneumonia (N= 24)	p
Antithrombotic therapy			
None, %	22.0 % (N=28)	45.8 % (N=11)	0.015
Prophylactic LMWH, %	65.4 % (N=83)	54.2 % (N=13)	0.296
Intermediate/treatment dose LMWH, %	12.6 % (N=16)	0 %	0.076
Thrombotic events, % (95%CI)	Overall: 5.5 % (2.2 - 11.0 %) (N=7)	0 % (0 - 14.2%)	0.004*
	ICU Non-ICU		
	15.2 % 2.1 % (0.3% (5.1 - 35.9%) -7.5%) (N=2) (N=5)		
	p = 0.013		
Bleeding events, % (95%CI)	Overall: 3.8% (1.2 - 8.6%) (N=5)		0.448*
	ICU Non-ICU		
	6.1 % 3.2% (0.7 - 20.2%) (0.7-9.0%) (N=3) (N=2)	0 % (0 - 14.2%)	
	p = 0.604		

*p value of Chi-Squared test for 3 categories (COVID-19 ICU and non-ICU and SARS-CoV-2-negative Pneumonia).
ICU: intensive care unit. LMWH: low molecular weight heparin.

Table 3. Baseline and peak laboratory parameters in COVID-19 patients and in those with SARS-CoV-2 negative pneumonia. Data are shown as mean ± SD.

	Baseline		Extreme value†		Normal range
	COVID-19	SARS-CoV-2-negative Pneumonia	COVID-19	SARS-CoV-2-negative Pneumonia	
Inflammation markers					
LDH, IU/L <i>p</i>	505 ± 213	336 ± 65 <i>< 0.001</i>	668 ± 385	381 ± 77 <i>< 0.001</i>	208 - 378
CRP, mg/dL <i>p</i>	8.2 ± 8.3	7.2 ± 6.4 <i>0.79</i>	11.1 ± 9.8	7.5 ± 6.9 <i>0.12</i>	0 - 1.0
IL-6, pg/mL <i>p</i>	ND	ND -	98.6 ± 304.7	16.0 ± 13.0 <i>0.065</i>	0 - 4.4
Routine hemostatic tests					
Platelets, x10 ⁹ /L <i>p</i>	216.2 ± 87.3	242.2 ± 89.0 <i>0.11</i>	194.9 ± 87.3	245.9 ± 89.1 <i>0.008</i>	150.0 - 450.0
PT, % <i>p</i>	91.8 ± 17.9	93.6 ± 12.6 <i>0.68</i>	79.8 ± 17.2	85.0 ± 13.3 <i>0.22</i>	75 - 100
aPTT, ratio <i>p</i>	1.06 ± 0.19	1.04 ± 0.12 <i>0.79</i>	ND	ND -	0.80 - 1.30
Fibrinogen, mg/dL <i>p</i>	457 ± 189	482 ± 150 <i>0.76</i>	514 ± 180	508 ± 153 <i>0.74</i>	150.0 - 400.0
D-dimer, mg/dL <i>p</i>	1,308 ± 2,042	556 ± 464 <i>0.001</i>	4,162 ± 9,492	690 ± 489 <i>< 0.001</i>	0 - 500
ISTH-DIC score					
Median, IQR <i>p</i>	0 (0 - 1)	0 (0 - 0.25) <i>0.024</i>	1 (0 - 2)	0 (0 - 1) <i>0.001</i>	□ 1

†Peak or minimum values of each laboratory parameter are shown, depending on its upward or downward tendency during COVID-19, respectively.

aPTT: activated partial thromboplastin time. CRP: C-reactive protein. IL-6: interleukin-6. LDH: lactate dehydrogenase. PT: prothrombin time.

O.D.: optical density.

P values were based on Mann-Whitney-U test or T-test.

Table 4. Thrombin generation parameters of plasma samples from pneumonia patients with or without COVID-19 and healthy controls. Values are expressed as median [IQR].

TGA parameter	Activator	COVID-19 (N= 127)	SARS-CoV-2 negative (N= 24)	<i>p</i>	Healthy subjects (N= 12) & pool*	<i>p</i> (vs COVID- 19)
Lag-time (min)	TF (5pM)	3.83 [3.33-4.50]	3.33 [3.00-3.79]	0.009	3.36 [2.69-4.50]	0.284
	Silica (1/16)	3.00 [2.50-3.33]	3.02 [2.67-3.36]	0.527	3.33 [2.59-3.43]	0.502
ETP (nM/min)	TF (5pM)	1263 [1101- 1488]	1506 [1235-1747]	0.005	1169 [1092-1732]	0.376
	Silica (1/16)	1390 [1205- 1610]	1556 [1304-1851]	0.011	1501 [1440-1732]	0.144
Peak (nM)	TF (5pM)	224 [179-256]	237.3 [206-269]	0.037	119 [92-236]	0.011
	Silica (1/16)	191 [166-222]	190.0 [172-225]	0.696	171 [157-240]	0.392
Tt Peak (min)	TF (5pM)	6.67 [6.00-7.67]	6.20 [5.33-6.86]	0.030	8.54 [5.36-11.67]	0.226
	Silica (1/16)	5.83 [5.17-6.50]	6.00 [5.40-6.70]	0.411	6.67 [5.60-6.85]	0.141
Vel Index (nM/min)	TF (5pM)	79.3 [56.6-103.3]	91.8 [70.8-111.9]	0.139	23.1 [13.8-90.6]	0.007
	Silica (1/16)	67.0 [55.1-84.1]	64.5 [58.3-83.9]	0.800	49.7 [45.6-87.5]	0.147
ratio ETP sTM/No	TF (5pM)	0.86 [0.76-0.91]	0.86 [0.82-0.89]	0.706	0.63 [0.52-0.89]	0.023

* Plasma pool of 100 blood donors; Abbreviations: sTM, soluble thrombomodulin; TF, Tissue factor; TGA, thrombin generation assay; Tt Peak, time to peak; Vel Index, velocity index. P values were based on Mann-Whitney-U test.

Table 5. Association between thrombin generation parameters (lag-time and ETP) induced by tissue factor (5 pM) and analytical data from tissue damage, cellular lysis, coagulation, and inflammation in COVID-19 patients. Data presented indicate the relationship between thrombin generation parameters and inflammatory markers, dichotomized according to median values, and also multiple linear regression analysis. The strength of correlations is indicated with a blue-red color scheme.

	Median (N)	LDH (IU/L)		CRP (mg/L)		IL-6 (pg/mL)		Fib (mg/dL)		D-D (mg/L)	
		<564 (62)	>546 (65)	<8.6 (71)	>8.6 (56)	<13.8 (75)	>13.8 (52)	<446 (66)	>446 (61)	<1.24 (63)	>1.24 (64)
Lag-time (min)	mean±SD	3.7±1.1	4.3±1.2	3.9±1.3	4.2±0.9	3.8±1.1	4.3±1.1	4.1±1.3	3.9±0.9	3.7±0.7	4.4±1.3
	<i>p</i>	0.022		0.01		0.004		0.969		<0.001	
	Correlation	0.247		0.218		0.397		0.068		0.407	
	Rho	0.247		0.218		0.397		0.068		0.407	
	<i>p</i>	0.007		0.021		<0.001		0.461		<0.001	
ETP (nM/min)	mean±SD	1350±320	1222±270	1267±330	1307±260	1264±300	1314±300	1235±340	1337±240	1354±280	1216±300
	<i>p</i>	0.016		0.19		0.368		0.022		0.019	
	Correlation	-0.241		0.024		-0.046		0.300		-0.288	
	Rho	-0.241		0.024		-0.046		0.300		-0.288	
	<i>p</i>	0.009		0.804		0.647		0.001		0.001	

Rho: -0.5 0 0.5



Rho: Spearman's Rho. ETP: Endogenous thrombin potential. LDH: Lactate dehydrogenase; CRP: C reactive protein; IL: Interleukin; O.D.: optical density; Fib: Fibrinogen; D-D: D-dimer. P values were based on Spearman's Rho correlations, and on Mann-Whitney-U test.

Table 6. Association between clinical outcomes and thrombin generation parameters obtained with tissue factor (5 pM) in samples from hospitalized COVID-19 patients.

	CURB-65		ICU		ARDS		Death		Thrombosis		Complication*	
	0-1 (N= 88)	≥ 2 (N= 39)	No (N= 94)	Yes (N= 33)	No (N= 88)	Yes (N= 39)	No (N= 117)	Yes (N= 10)	No (N= 120)	Yes (N= 7)	No (N= 86)	Yes (N= 41)
Lag-time	3.8±0.9	4.5±1.4	3.9±1.1	4.4±1.2	3.9±1.0	4.4±1.3	4.0±1.2	4.1±0.9	3.9±1.0	5.8±2.3	3.8±0.9	4.5±1.4
<i>p</i>	0.004		0.020		0.018		0.641		0.009		0.020	
ETP	1340±261	1158±341	1352±284	1092±255	1332±290	1178±293	1302±297	1082±251	1312±272	808±355	1382±258	1080±277
<i>p</i>	0.003		<0.001		0.008		0.011		0.001		<0.001	

Abbreviations; ARDS, acute respiratory distress syndrome. *Complications include ARDS, Thrombosis, Hemorrhage, or Death. P values were based on Mann-Whitney-U test.

Figures

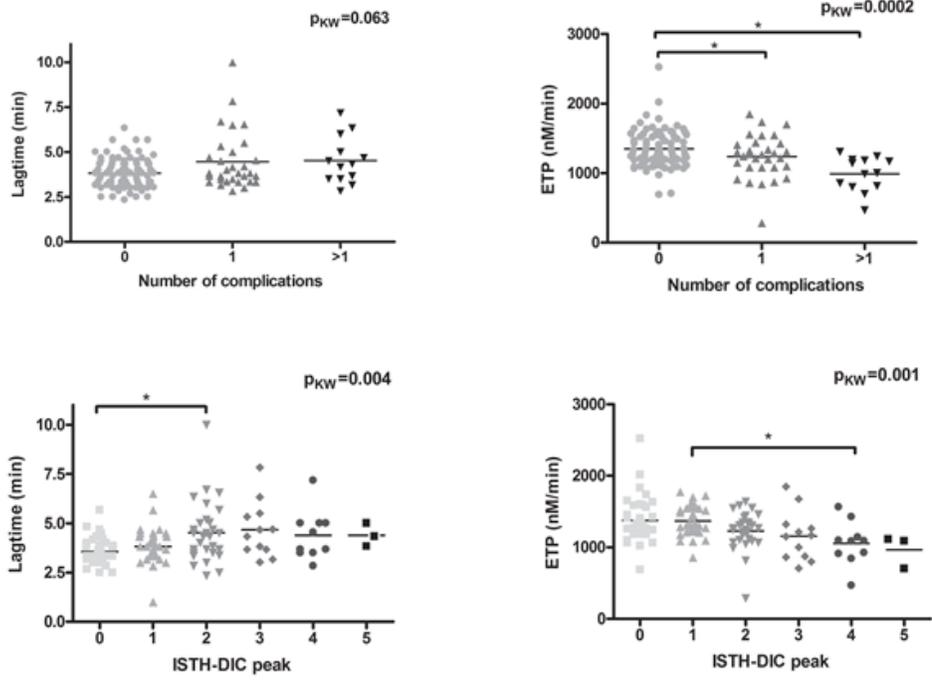


Figure 1

Association of lag-time and ETP values of COVID-19 patients with the number of complications (A) and ISTH-DIC scores (B). p_{KW} : p value based on Kruskal Wallis test comparing all groups; * $p < 0.05$ based on Mann-Whitney-U test comparing pairs.

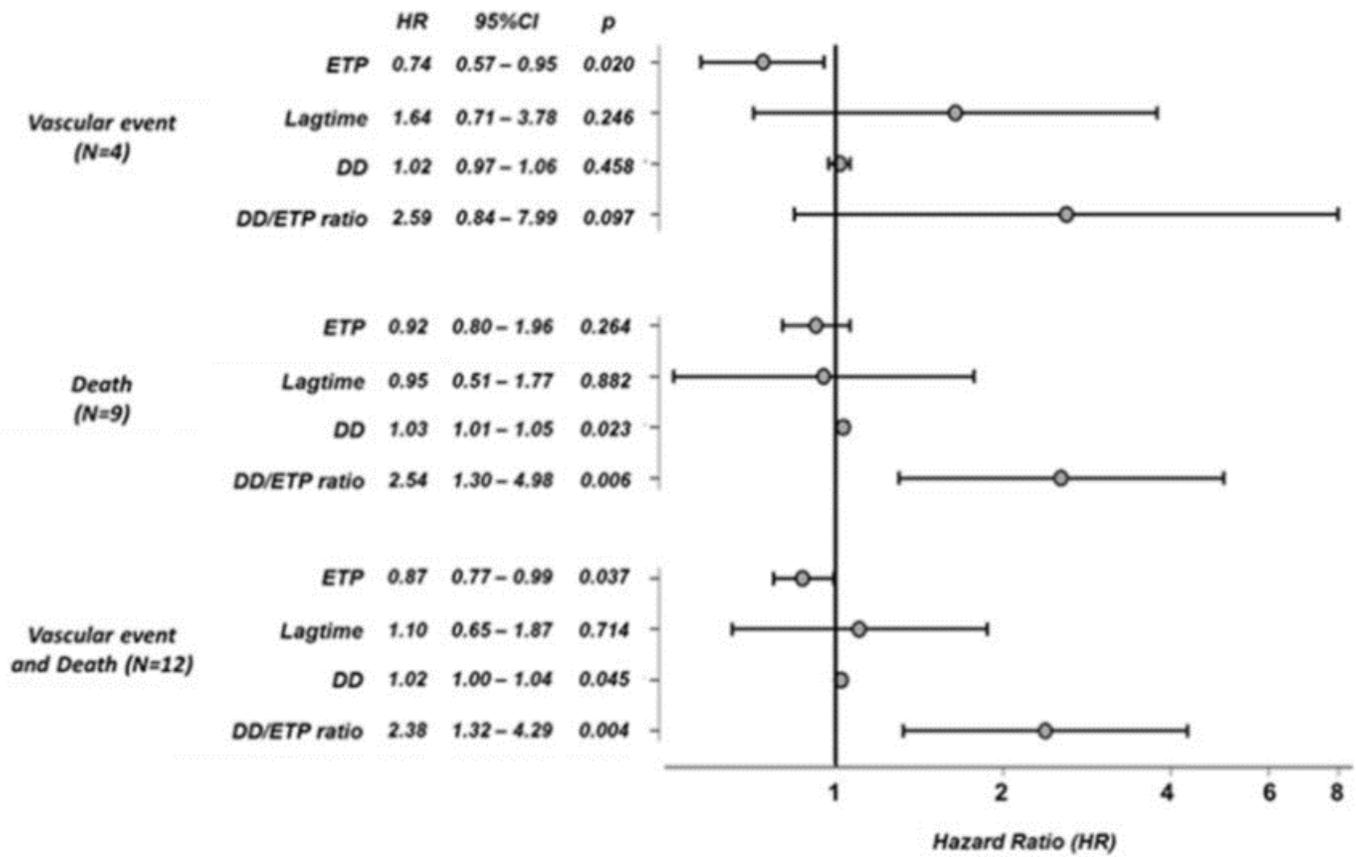


Figure 2

Cox regression analysis of thrombin generation parameters, D-dimer and the D-dimer/ETP ratio in COVID-19 patients. Square root transformation of ETP and D-dimer and logarithmic transformation of D-dimer/ETP ratio were performed for predictive analysis. 95% confidence interval and p value are shown for each Hazard Ratio. DD: D-dimer. HR: Hazard ratio.

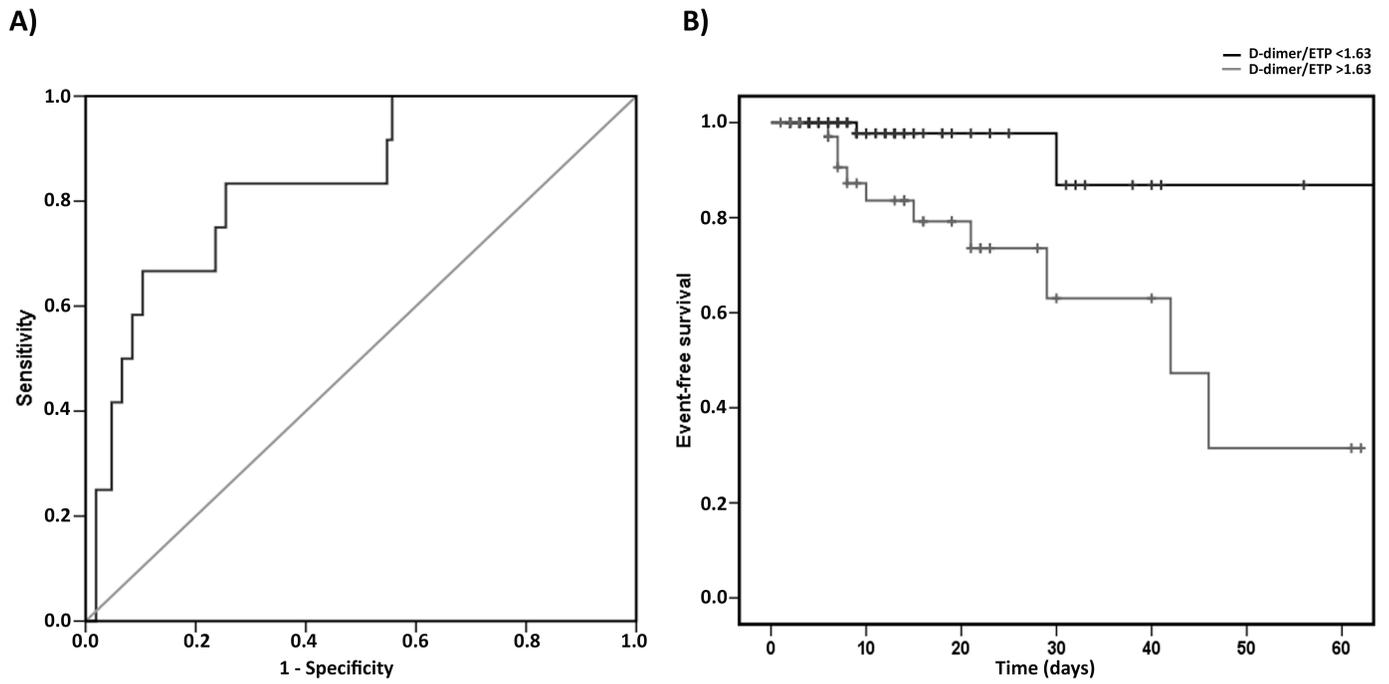


Figure 3

Ratio D-dimer/ETP as predictor for adverse events (vascular events or death) in COVID-19 patients. A) ROC curve; B) Kaplan-Meier curve.

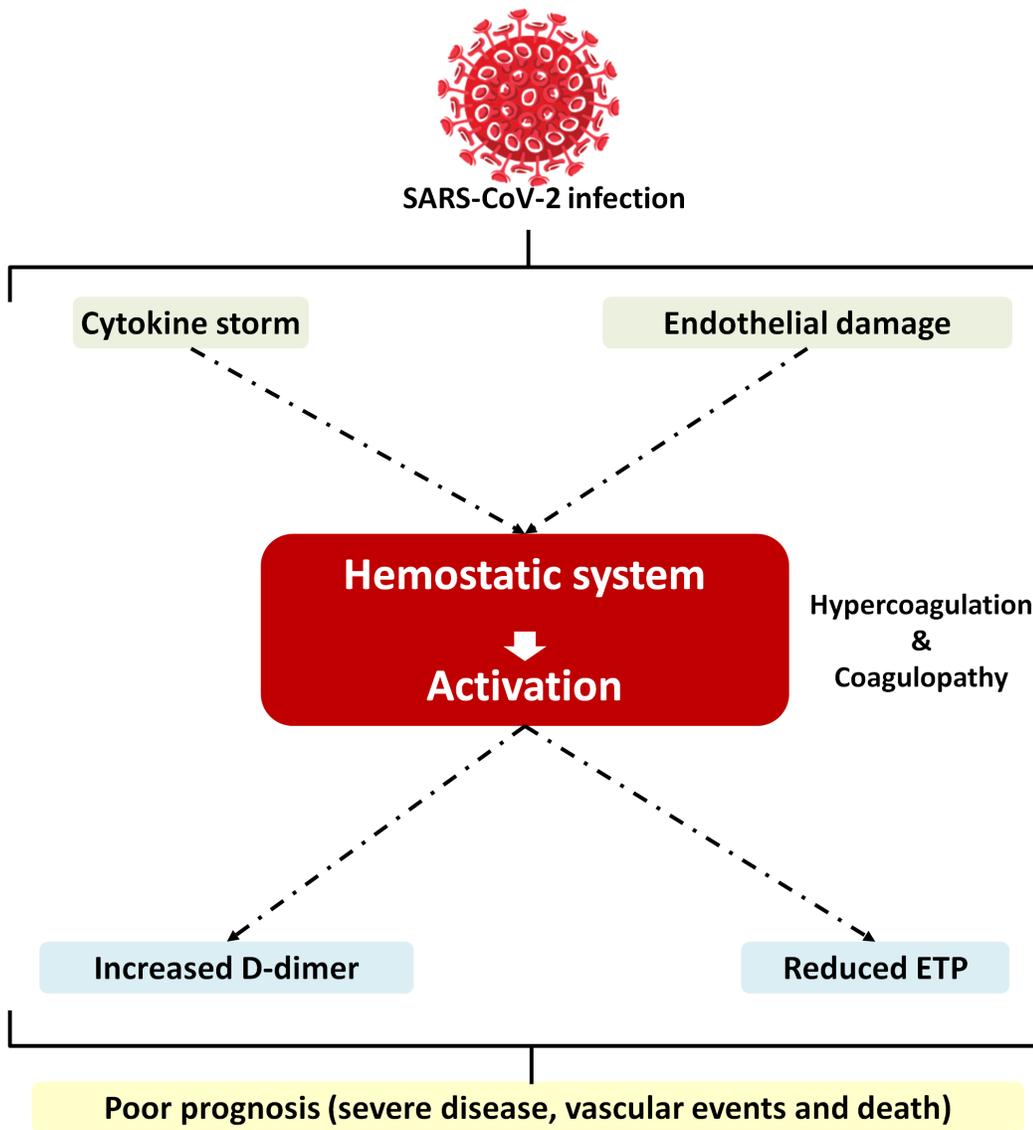


Figure 4

Schematic representation of potential insults associated to SARS-CoV-2 infection and the consequences on the hemostatic system.

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