

More Severe Hypercoagulable State in Acute COVID-19 Pneumonia as Compared With Other Pneumonia

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Abstract

Objective: To conduct a comprehensive evaluation of coagulation profiles—via traditional and whole blood thromboelastometry tests—in coronavirus disease 2019 (COVID-19)—positive vs COVID-19—negative patients admitted to medical wards for acute pneumonia.

Patients and Methods: We enrolled all consecutive patients admitted to internal medicine wards of Padova University Hospital between 7 March and 30 April, 2020, for COVID-19—related pneumonia (cases) vs non—COVID-19 pneumonia (controls). A group of healthy individuals acted as baseline for thromboelastometry parameters.

Results: Fifty-six cases (mean age, 64 ± 15 years; male/female, 37/19) and 56 controls (mean age, 76 ± 11 years; male/female, 35/21) were enrolled. Cases and controls exhibited markedly hypercoagulable thromboelastometry profiles vs healthy individuals, mainly characterized by a significantly shorter propagation phase of coagulation (clot formation time) and significantly increased maximum clot firmness ($P < .001$ for all comparisons). Patients with COVID-19 pneumonia had significantly shorter clot formation time and higher maximum clot firmness ($P < .01$ and $P < .05$, respectively, for all comparisons) than did controls.

Conclusion: Patients admitted to internal medicine wards for COVID-19 pneumonia presented a markedly prothrombotic state, which seems peculiar to COVID-19 rather than pneumonia itself.

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The novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) infection that spread rapidly worldwide from China in late December 2019 has been linked to several coagulation abnormalities. Tang et al¹ reported that elevated D-dimer levels, prolonged prothrombin time, and activated partial thromboplastin time were common findings in patients with coronavirus disease 2019 (COVID-19) and correlated with a worse prognosis. Considerably elevated D-dimer plasma levels in patients with a more severe infection were also confirmed by 2 pooled data analyses.^{2,3} Interestingly, we and 2 other Italian groups recently reported hypercoagulable whole blood profiles in patients with

COVID-19 admitted to intensive care units for acute respiratory failure.⁴⁻⁶ The hypercoagulability observed in patients with COVID-19 may be responsible for the high prevalence of both arterial and venous thrombotic events.^{7,8} Moreover, the association between coagulation activation and more severe infection may explain the better outcome observed in patients undergoing anticoagulant therapy (eg, heparin).⁹ To our knowledge, no study published in the literature so far has conducted a comprehensive evaluation of coagulation profiles as it relates to the comparison between patients with and without COVID-19 pneumonia. Therefore, we aimed to study traditional and whole blood thromboelastometry profiles—via a rotation thromboelastometry

(ROTEM) sigma apparatus (Instrumentation Laboratory — Werfen)—in a group of patients consecutively admitted to internal medicine wards of Padova University Hospital for acute pneumonia: cases were patients with COVID-19 pneumonia and controls were patients with pneumonia from different etiology.

PATIENTS AND METHODS

All consecutive patients admitted between 7 March and 30 April, 2020, to COVID-19 medical wards of Padova University Hospital with a confirmed diagnosis (ie, positive nasopharyngeal swab results) of acute COVID-19 pneumonia were considered for enrollment. An equal number of patients admitted to internal medicine wards (non—COVID-19 area) over the same period of time for acute pneumonia and with a negative COVID-19 nasopharyngeal swab acted as controls. For the diagnosis of acute pneumonia, we referred to the National Institute for Health and Care Excellence pneumonia guideline.¹⁰ In particular, the suspicion of pneumonia was prompted by the onset over the past 3 days of at least 2 symptoms among the following: cough, newly expressed fatigue, pain while breathing, anosmia, myalgia, or confusion; and the presence of at least 2 signs among the following: temperature above 38°C, respiratory rate greater than 20 breaths/min, heart rate greater than 100 beats/min, or focally depressed or altered breathing sounds or dullness to percussion. The diagnosis was confirmed in each patient by chest radiography. Exclusion criteria were as follows: pregnancy, known congenital discoagulopathies, age less than 18 or more than 90 years, Child's C liver disease, severe chronic kidney disease, active cancer, preexisting hematological disorders, and ongoing anticoagulant therapy. It bears noting that during the enrollment of our study population, it was not standard practice in our hospital to initiate anticoagulant therapy in patients with COVID-19 upon admission. Thus, after obtaining informed consent from each enrolled patient, we collected the following data within 6 hours of admission: (1) demographic characteristics, clinical data, and Sequential Organ Failure Assessment score; (2) 2 BD vacutainers of venous blood (Becton Dickinson) with 109 mmol/L sodium

citrate (3.2% sodium citrate). A group of healthy individuals age- (± 3 years) and sex-matched with patients with COVID-19 acted as baseline for ROTEM parameters. The study was conducted in adherence to the Declaration of Helsinki. The protocol was approved by the Padova University Hospital ethics committee.

Laboratory Tests

Blood cell counts, traditional coagulation parameters (ie, prothrombin time/international normalized ratio, activated partial thromboplastin time, fibrinogen level, D-dimer level, and antithrombin level), C-reactive protein level, and procalcitonin level were measured using standard laboratory methods. Whole blood thromboelastometry profiles were obtained within 3 hours of blood collection via the completely automated ROTEM sigma apparatus (Instrumentation Laboratory — Werfen) according to the manufacturer's recommendations. The INTEM and EXTEM assays (evaluation of intrinsic and extrinsic coagulation pathways) and FIBTEM assay (evaluation of fibrinogen contribution to blood clot) were performed for each enrolled patient. The following parameters were assessed: (1) clotting time (CT; in seconds), the time from the beginning of the coagulation analysis until an increase in amplitude of the thromboelastographic trace of 2 mm; CT evaluates the activation phase of the clot formation; (2) clot formation time (CFT; in seconds), the time elapsed for an increase in amplitude of the thromboelastogram from 2 to 20 mm; CFT explores the propagation phase of the clot formation; (3) maximum clot firmness (MCF; in millimeters), the maximum amplitude in millimeters reached in the thromboelastogram; and (4) maximum lysis (in percentage), the maximum percentage of clot lysis. A *hypercoagulable thromboelastometry profile* was defined as shorter CTs and/or CFTs and/or higher MCF compared with controls as well as healthy individuals.¹¹

Statistical Analyses

The sample size calculation was based on pilot observations and the following assumptions: (1) expected mean increase in MCF to identify hypercoagulable profiles 3 ± 4 mm or more¹⁰; (2) power, 90%; (3) α , .05. Therefore, we

TABLE 1. Patients' Characteristics and Laboratory Data^{a,b,c}

Characteristic	COVID-19 pneumonia	Non-COVID-19 pneumonia	P value
No. of patients	56	56	—
Age (y)	64±15	76±11	.13
Sex: M/F	37 (66)/19 (34)	35 (63)/21 (37)	.69
BMI (kg/m ²)	30±4	27±6	.24
SOFA score	2±1	3±1	.62
WBC count (×10 ⁹ /L)	6.83±3.20	11.50±4.41	<.001
Lymphocyte count (×10 ⁹ /L)	1.13±1.60	1.20±0.63	.70
Hemoglobin level (g/L)	119±26	121±24	.76
Platelet count (×10 ⁹ /L)	277±131	274±89	.90
PT (%)	87±20	81±15	.09
INR	1.2±0.4	1.3±0.3	.13
aPTT (s)	26±5	28±5	.12
D-dimer level (ng/L)	1079±666	1296±8	.55
Fibrinogen level (mg/dL)	451±168	488±198	.33
Antithrombin level (%)	103±16	102±12	.95
C-reactive protein (mg/L)	60±56	114±77	<.001
Procalcitonin level (ng/mL)	1.5±6.2	4.4±6.8	.03

^aaPTT = activated partial thromboplastin time; BMI = body mass index; COVID-19 = coronavirus disease 2019; F = female; INR = international normalized ratio; M = male; PT = prothrombin time; SOFA = Sequential Organ Failure Assessment; WBC = white blood cell.

^bSI conversion factors: To convert mg/dL values to mmol/L, multiply by 0.0259; to convert ng/mL values to nmol/L, multiply by 2.5.

^cData are presented as mean ± SD or as No. (percentage)

needed 2 groups (COVID-19 vs non-COVID-19 pneumonia) of at least 45 patients each. Categorical variables were expressed as counts and

TABLE 2. ROTEM Parameters^{a,b}

Parameter	COVID-19 pneumonia	Non-COVID-19 pneumonia	P value	Healthy individuals
INTEM				
CT (s)	174±17	178±33	.49	176±25
CFT (s)	50±15	59±15	.01	84±20 ^c
MCF (mm)	69±7	66±8	.04	58±4 ^c
ML (%)	1-2	2-3	—	0-5
EXTEM				
CT (s)	66±9	70±11	.08	65±12
CFT (s)	48±15	62±16	<.001	102±17 ^c
MCF (mm)	71±6	69±6	.03	56±5 ^c
ML (%)	1-2	2-3	—	0-5
FIBTEM				
MCF (mm)	30±7	27±8	.03	13±4 ^c

^aCFT = clot formation time; COVID-19 = coronavirus disease 2019; CT = clotting time; MCF = maximum clot firmness; ML = maximum lysis.

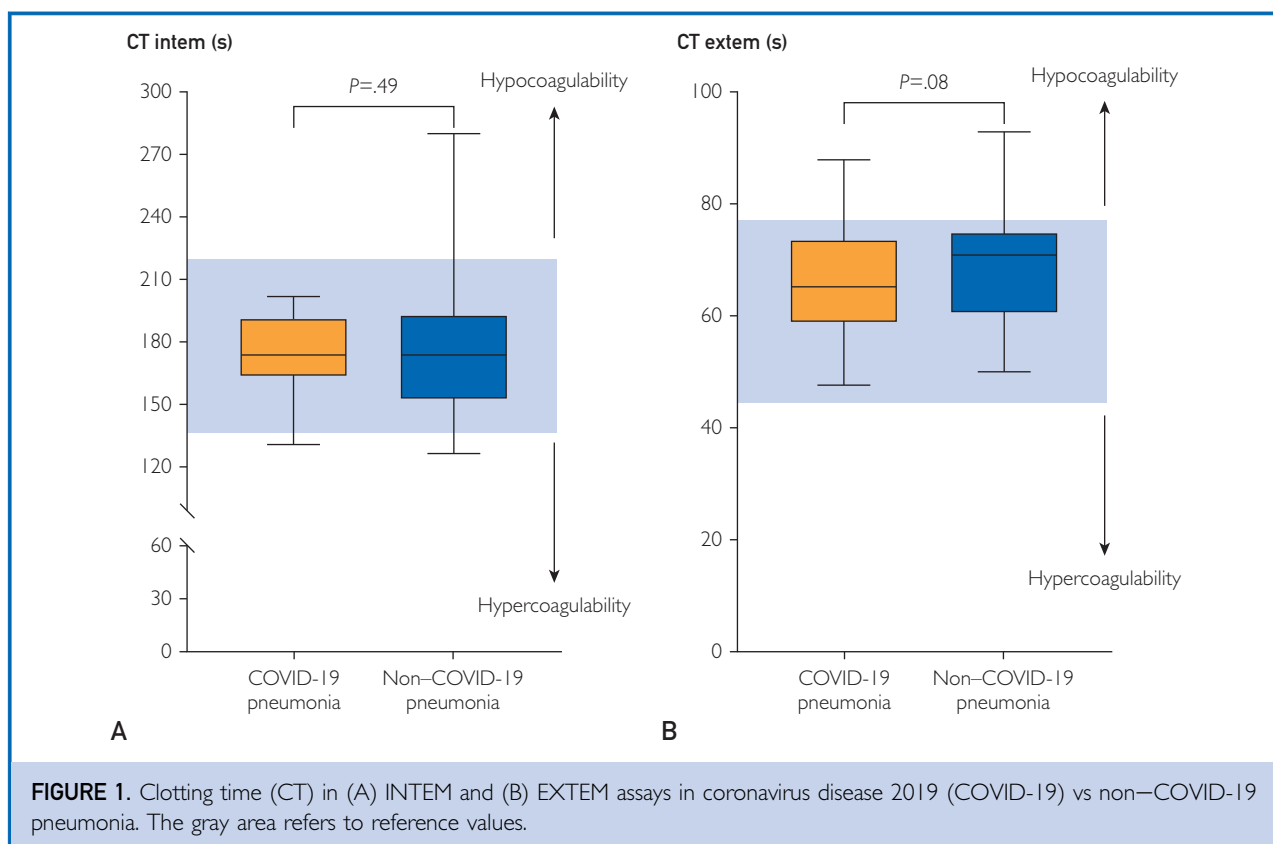
^bData are expressed as mean ± SD or as range.

^cP<.001 vs COVID-19 and non-COVID-19 pneumonia.

percentages. Continuous variables were expressed as mean ± SD. The normality assumption was assessed using the Shapiro-Wilk normality test. The chi-square test and Student *t* test were used, where appropriate, to compare cases and controls. Lower and upper values of the reference ranges for ROTEM parameters were determined in the population of healthy individuals using the 2.5th and 97.5th percentile, respectively. A *P* value less than .05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software Inc.).

RESULTS

Of the 63 patients consecutively admitted to our hospital for COVID-19 pneumonia, 56 (89%) (mean age, 64±15 years; male/female, 37/19) were enrolled; 4 patients were excluded for ongoing anticoagulant therapy at the time of admission, 2 for active cancer, and 1 for acute renal failure. Of the 64 patients hospitalized in non-COVID-19 wards during the same period of time for non-COVID-19 pneumonia, 56 (88%) (mean age, 76±11 years; male/female, 35/21) were enrolled; 3 patients were excluded for age more than 90 years, 2 for ongoing anti-coagulant therapy at the time of admission, 1 for active cancer, 1 for end-stage liver, and 1 for acute renal failure. Patients' characteristics for each group are reported in Table 1. No substantial differences were observed between groups as they relates to the main clinical and laboratory data. In particular, traditional coagulation parameters were similar between cases and controls. Only white blood cell count (*P*<.001), C-reactive protein level (*P*<.001), and procalcitonin level (*P*=.03) were significantly higher in non-COVID-19 pneumonia than in COVID-19 pneumonia. ROTEM parameters (see Table 2) revealed a significantly reduced CFT in INTEM and EXTEM in cases and controls vs healthy individuals (*P*<.001 for both comparisons). Moreover, MCF in INTEM, EXTEM, and FIBTEM was significantly higher in cases and controls than in healthy individuals (*P*<.001 for all comparisons). We observed no significant differences between CT in INTEM or EXTEM assays in COVID-19 pneumonia and non-COVID-19 pneumonia (Figure 1A and B). Notably, CFT in INTEM and EXTEM assays was significantly shorter in cases than in controls (*P*=.01 and *P*<.001,



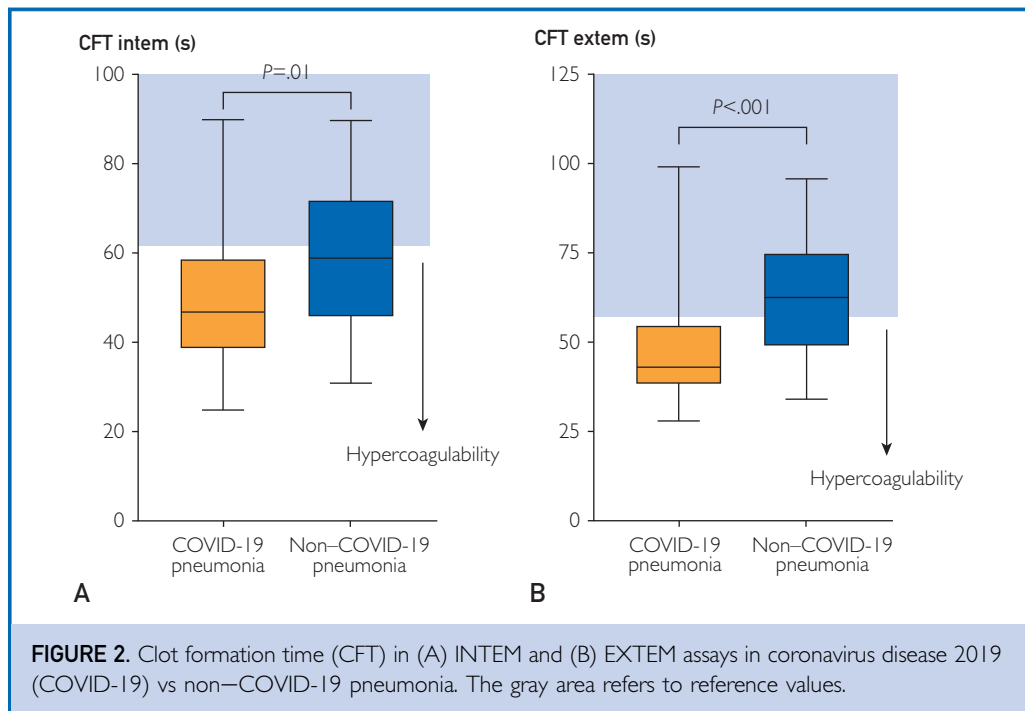
respectively) (Figure 2A and B). Moreover, MCF in INTEM, EXTEM, and FIBTEM assays was significantly higher in cases than in controls ($P=.04$, $P=.03$, and $P=.03$, respectively) (Figure 3A-C).

DISCUSSION

The results of our study indicate that patients with COVID-19 admitted to medical wards for acute pneumonia had markedly hypercoagulable profiles on whole blood thromboelastometry, as compared with COVID-19-negative patients with pneumonia, mainly characterized by a significantly shorter propagation phase of the clot formation and significantly increased clot firmness. These findings appear to suggest that the markedly hypercoagulable profiles on whole blood thromboelastometry in patients with COVID-19 pneumonia vs controls may be attributable to the SARS-CoV-2 infection specifically, rather than pneumonia itself. Although several articles,

including one by our group, have previously reported COVID-19-related hypercoagulability in patients admitted to intensive care units,⁴⁻⁶ the present study focuses more closely on the comparison of coagulation profiles between patients with COVID-19 acute pneumonia and patients without COVID-19 acute pneumonia. Furthermore, although previous reports found that traditional coagulation parameters (eg, D-dimer level, factor VIII level, fibrinogen level, and von Willebrand factor level) were able to identify hypercoagulable profiles in patients with COVID-19,^{6,12} we observed no significant differences between cases and controls in our study population as it pertains to traditional coagulation tests. Moreover, as previously reported,¹³ we found no evidence of consumptive coagulopathy in patients with COVID-19.

From a clinical standpoint, the marked increase in coagulation capability observed in our study may account for the high risk



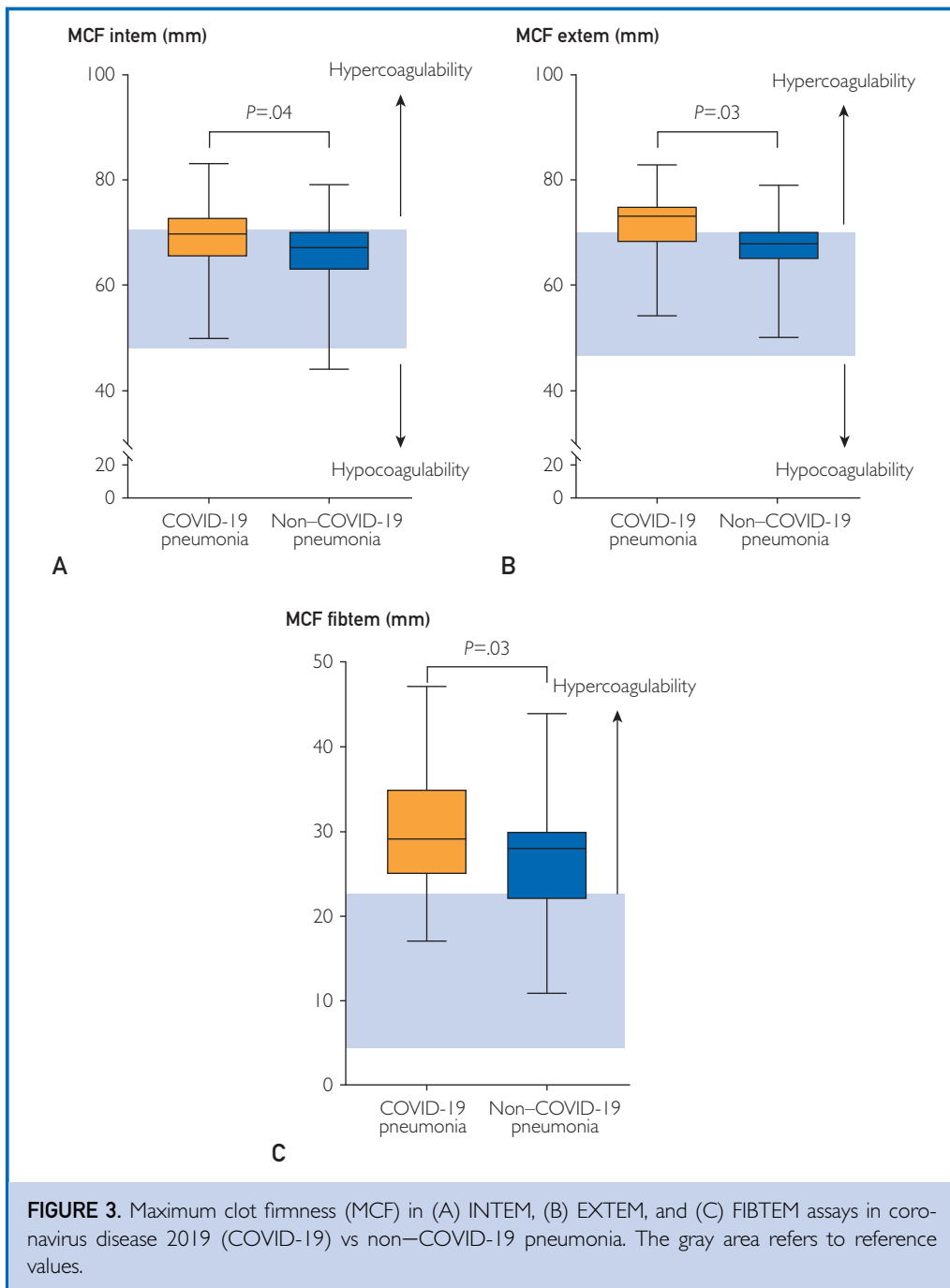
of thromboembolic events observed in patients with COVID-19 acute pneumonia.^{7,8} Hence, the beneficial effect of administering low-molecular-weight heparin, either at prophylactic or at therapeutic dosages, is the reduction in the incidence of thrombotic events and mortality.^{9,14} Another clinical implication of hypercoagulability—but specifically, the significant increase in clot firmness in the FIBTEM assay—stems from the markedly increased capability of fibrin to polymerize, which may lead to microcirculation thrombosis (ie, occlusive microthrombi in pulmonary small vessels) and fibrin deposition in alveolar and interstitial lung spaces, ultimately resulting in the development of acute respiratory failure in patients with COVID-19.

Some of the limitations of our study must be addressed. First, the lack of standardization of the thromboelastometry device limits data comparison across different laboratories. In this regard, the use of a fully automated tool such as ROTEM sigma reduces intra- and intertest variability and thus provides more standardized and comparable data. Furthermore, the use of the ROTEM sigma, a closed, cartridge-based, fully automated viscoelastic

testing system, allows us to safely manage hazardous samples such as of COVID-19, thus minimizing the risk of contamination.¹⁵ Second, despite our best efforts to closely match our control group to cases, the former ended up being rather heterogeneous, comprising both patients with bacterial and viral pneumonia. Finally, the relatively low sample size did not allow to adjust the statistical analyses for specific confounding factors (eg, age, obesity, and Sequential Organ Failure Assessment score), which we know from the literature may single-handedly yield hypercoagulable ROTEM profiles.¹⁶

CONCLUSION

Our findings indicate that patients with COVID-19 admitted to internal medicine wards present a markedly hypercoagulable state, which appears to be a peculiarity of SARS-CoV-2 infection rather than pneumonia itself. Larger prospective studies are warranted to evaluate the possible association between the observed prothrombotic state and clinical outcomes such as the development of thrombotic complications or worsening of clinical conditions (ie, admission to the intensive care unit, intubation, or death).



Abbreviations and Acronyms: CT = clotting time; CFT = clot formation time; COVID-19 = coronavirus disease 2019; MCF = maximum clot firmness; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

Potential Competing Interests: The authors report no competing interests.

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