ORIGINAL ARTICLE

European Journal of Haematology | WILEY

COVID-19 coagulopathy: An in-depth analysis of the coagulation system

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Abstract

Background: Abnormal coagulation parameters have been reported in COVID-19-infected patients. Although the underlying mechanism of COVID-19 coagulopathy remains unknown, it has been suggested to be a form of disseminated intravascular coagulation (DIC).

Objectives: The aim of our study was to analyze the coagulation parameters of patients with COVID-19, determine whether coagulation factors consumption occurs and identify potential prognostic biomarkers of the disease.

Patients/Methods: Blood samples from hospitalized patients with COVID-19 pneumonia were collected. We performed basic coagulation tests and quantification of coagulation factors and physiological inhibitor proteins. Laboratory data were compared with clinical data and outcomes.

Results: The study involved 206 patients (63.6% male). D-dimer was particularly elevated (median 450 ng/mL; IQR 222.5-957.3). Free protein S levels were below the normal range (median 56.6%; IQR: 43.6-68.9), and factor VIII showed an increasing trend (median 173.4%; IQR: 144.1-214.9). However, all coagulation factors were within normal limits. We found no correlation between abnormal coagulation parameters and thrombosis, except for higher D-dimer (HR 1.99; 95% CI 1.3-3.1; $P = .002$).

Conclusions: COVID-19 is associated with coagulopathy that correlates with poor prognosis. However, we did not demonstrate a consumption of coagulation factors, as seen in DIC.

KEYWORDS
coagulation factors, coagulopathy, COVID-19, D-dimer, SARS-CoV-2 infection

1 | INTRODUCTION

In late December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Wuhan, China.[1,2] Since then, this virus has rapidly spread worldwide, with more than 15 million confirmed cases of COVID-19, the clinical condition associated with this infection, and more than 630,000 deaths at the time of writing.[3]

The pathophysiology of COVID-19 and the underlying mechanism of its clinical manifestations remain unclear.[4] However, several studies have reported abnormal coagulation parameters, notably in patients with COVID-19 associated pneumonia and acute respiratory distress syndrome (ARDS).[5,6] Among the hemostatic system alterations identified, increased D-dimer levels seem to constitute an independent biomarker of poor prognosis in COVID-19, as first stated in early reports from China and confirmed in different series of patients worldwide.[7] Additionally, these reports suggested that COVID-19 coagulopathy might be a form of disseminated intravascular coagulation.

In line with the hypothesis that coagulopathy may play a role in COVID-19 pathogenesis, recent data suggest an increased risk of developing deep vein thrombosis (DVT) and pulmonary embolism (PE) among patients with severe COVID-19 infection,[8,9] especially those admitted in intensive care units (ICU), despite adequate thromboprophylaxis. Furthermore, postmortem studies have highlighted the presence of pulmonary microthrombi and capillarostasis.[10]

Despite all the efforts of the scientific community to understand COVID-19 infection and its associated coagulopathy, there is still a lot to clarify on its mechanism and the way the hemostatic system plays a role on the development of this disease and its clinical complications.

The aim of our study was to analyze the coagulation parameters of patients with COVID-19 pneumonia admitted to our hospital, determine whether coagulation factors consumption occurs and identify potential prognostic biomarkers of this new disease.

2 | METHODS

We conducted a retrospective cohort study performed at Gregorio Marañon Hospital in Madrid. Consecutive blood samples from hospitalized adult patients with COVID-19 pneumonia were collected from our Hemostasis laboratory between April 3 and May 3, 2020. According to the local COVID-19 protocol, blood samples were obtained daily from every hospitalized patient with COVID-19, including one to perform a basic coagulation test. Thus, the samples collected for our study were either diagnostic or follow-up available samples from inpatients with COVID-19 pneumonia. We collected an average of 10 samples per day that were selected on a random basis. One sample of blood was obtained from each patient.

Inclusion criteria were blood samples from patients with COVID-19 aged 18 years or older hospitalized in our center, regardless the time from admission. COVID-19 diagnosis was defined by positive PCR in nasopharyngeal swab or by radiologic and analytical findings


Novelty Statement

1. The new aspect of our study is the exhaustive analysis of all the factors involved in the coagulation cascade to asses whether a consumption coagulopathy is present in COVID-19-infected patients.
2. COVID-19 coagulopathy does not seem to be a form of disseminated intravascular coagulation, as we did not demonstrate a consumption of coagulation factors in our cohort.
3. Clinical management and scoring system of COVID-19 coagulopathy should be different from that of classical DIC.

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highly suggestive of the disease. Patients receiving vitamin K antagonists (VKAs) <10 days prior to the blood sample withdrawal were excluded to avoid interference with coagulation test results. This study was approved by our Institutional Ethics Committee, and it was executed along with the international ethical recommendations for conducting research in humans following the latest revision of Declaration of Helsinki.

For each sample, we conducted a complete analysis of coagulation parameters. Demographic, clinical, and routine laboratory data were obtained from the hospital electronic patient record using a standard data collection form. Routine laboratory data, according to our local COVID-19 protocol, included cell blood count, biochemistry, and acute phase reactants such as C-reactive protein, procalcitonin, ferritin, and interleukin 6. For each subject, clinical and routine laboratory data were collected at the time the hemostasis testing was performed.

All the coagulation parameters were analyzed in the Hemostasis Laboratory of the Hematology department. Assays included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, D-dimer and quantification of coagulation factors, and physiological inhibitor proteins (protein C, free protein S, and antithrombin). The PT, APTT, and fibrinogen assays were determined on the ACL Top 700 analyzer using HemosIL® RecombiPlasTin 2G, HemosIL® SyntASil, and HemosIL® Fibrinogen C reagents, respectively. D-dimer levels were measured in ng/mL Fibrinogen Equivalent Units (FEU) with the HemosIL® D-Dimer assay on the ACL Top 700 analyzer (Instrumentation Laboratory). Coagulation factors (II, V, VII, VIII, IX, X, XI, and XII) were determined using their corresponding HemosIL® reagents on the ACL Top 500. Physiological inhibitor proteins were assessed using HemosIL® Protein C, HemosIL® Free Protein S, and HemosIL® Liquid Antithrombin reagents on the ACL Top 500.

Qualitative variables are presented using frequency distribution and percentages. Quantitative variables are presented using the mean and the standard deviation (SD) if they are normally distributed or the median and the interquartile range (IQR) if they follow a non-normal distribution. Normal distribution was assessed using the Kolmogorov-Smirnov test. The comparison between qualitative
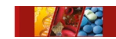
TABLE 1 Demographics, clinical, laboratory, and coagulation parameters of COVID-19-infected patients

Parameters	Normal values	Results (n = 206)	Survivors (n = 188)	Non-survivors (n = 18)	P value
Demographics and clinical parameters					
Age (y)	—	63.6 ± 13.4	62.4 ± 12.9	76.0 ± 12.3	<.001*
Sex (male/female)	—	131/75	120/68	11/7	.81
Comorbidities (yes/no)	—	161/45	143/45	18/0	.015*
DIC (yes/no)	—	11/195	7/181	4/14	.001*
Routine laboratory tests					
Hemoglobin (g/dL)	12-16	13 (11.5-14.2)	13.1 (12.1-14.3)	11.5 (10.2-13.7)	.021*
Platelets (x10³/μL)	140-400	279.69 (186.5-365)	262 (190-370)	203 (104-365.7)	.013*
Leucocytes (x10³/μL)	4-10	7.2 (5.4-9.9)	6.9 (5.3-9.5)	9.9 (7.8-13.6)	.001*
Neutrophils (x10³/μL)	1.8-7.5	5.5 (3.3-8.1)	5.3 (3.2-7.9)	8.6 (6.4-12.8)	<.001*
Lymphocytes (x10³/μL)	1.3-3.5	1 (0.4-0.8)	1 (0.7-1.4)	0.6 (0.4-0.8)	.007*
C-reactive protein (mg/dL)	0-0.5	2.9 (0.8-8.6)	2.8 (1.1-8.7)	8.4 (4.02-26.02)	.043*
Procalcitonin (μg/L)	0-0.5	0.07 (0.03-0.14)	0.06 (0.03-0.13)	0.11 (0.05-0.38)	.011*
Ferritin (μg/L)	5-204	710 (404-1389)	731 (405-1425)	763 (598.2-1354.5)	.331
Interleukin 6 (pg/mL)	0-4.3	34.4 (7.6-90.4)	32.5 (6.6-60.6)	139.2 (80.3-195.5)	<.001*
Coagulation parameters tests					
PT (s)	10.5-13.5	12.8 (12.1-13.6)	12.7 (12-13.4)	14 (12.7-15.6)	<.001*
INR	0.8-1.2	1.15 (1.09-1.23)	1.13 (1-1.2)	1.25 (1.2-1.4)	<.001*
APTT (s)	27-38	29.7 (26.8-32.6)	29.5 (27.3-32.5)	29.9 (26.4-30.8)	.181
Fibrinogen (mg/dL)	200-400	571.2 ± 187.7	575 ± 183.7	526.9 ± 226.5	.389
D-dimer (ng/mL)	0-250	450 (222.5-957.3)	385 (214-916.8)	1472.5 (390.8-3238.5)	.004*
Free Protein S (%)	70-140	56.6 (43.6-68.9)	56.1 (43.7-67.8)	50.7 (38.9-59.9)	.477
Protein C (%)	70-140	123 (105-155)	128 (107-159.3)	107 (76.5-119.8)	.001*
Antithrombin (%)	70-140	105 (92-117.3)	107 (93-118)	86 (69-101.3)	.008*
Factor II (%)	60-140	99.7 (87.7-110.9)	102.3 (89.4-110.9)	82.7 (75-94)	<.001*
Factor V (%)	60-140	111.6 (94.4-135.8)	111.6 (92.2-135.8)	115.9 (103.8-138.6)	.460
Factor VII (%)	60-140	81.7 (68.3-100.7)	81.9 (69.5-102.4)	66.2 (51.7-76.2)	.02*
Factor VIII (%)	50-200	173.4 (144.1-214.9)	173.4 (140.2-215.4)	167.7 (143.2-181.8)	.105
Factor IX (%)	60-140	142.8 (122.6-170.6)	142.8 (124.2-169.9)	147.1 (123.5-178.1)	.522
Factor X (%)	60-140	109.1 (92.9-126.8)	111.1 (98.1-128)	86.3 (79.4-112.9)	.007*
Factor XI (%)	60-140	125.8 (104.2-147.6)	128.7 (107.1-148.6)	147.1 (123.5-178.1)	.522
Factor XII (%)	60-140	115.1 (87.1-149.1)	119 (92.5-153.9)	110 (91.8-137.3)	.21

Note: The variable age and fibrinogen are normally distributed. The remaining quantitative variables follow a non-normal distribution. Data are presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for non-normally distributed variables.

Abbreviations: APTT, Activated partial thromboplastin time; DIC, disseminate intravascular coagulopathy; INR, International normalized ratio; PT, Prothrombin time.

*indicates statistically significant values.

variables was analyzed using chi-squared test and Fisher's exact test. For quantitative variables, the *t* test or the Mann-Whitney *U* test was performed depending on the normality of the variable. We used Spearman correlation to compare two quantitative variables. A multivariate analysis was performed using Cox proportional hazards model. A two-tailed *P*-value < .05 was considered to be statistically significant for all analysis. Data were analyzed using IBM SPSS Statistics for Mac (version 24; IBM Corp) and GraphPad Prism (version 8.4.3; GraphPad Software).

3 | RESULTS

3.1 | Characteristics of the sample

A total of 206 hospitalized patients with COVID-19 pneumonia were analyzed. Mean age of the sample was 63.6 (SD 13.4) years, and 63.6% of the patients were male (Table 1). Comorbidities were present in 161 patients (78.2%), including cardiovascular and respiratory diseases, cancer, chronic liver and kidney diseases, DVT

history, and others. Median days of hospitalization until the haemostasis testing were five days (IQR 2-10). At the moment of blood sample collection, 26 patients were admitted in the ICU (12.6%). At the time of writing, 158 patients (76.7%) had been discharged from hospital, 30 (14.6%) remained hospitalized and 18 (8.7%) had died. Median hospitalization time was 16 days (IQR 10-28.3).

The majority of patients (66%) were receiving low-flow oxygen therapy at the time of blood sample collection, either via nasal cannula (49.5%) or via non-rebreather mask (16.5%). High-flow oxygen therapy was used in 7.3% of patients, and 7.3% were on mechanical ventilation. A total of 40 patients (19.4%) did not require oxygen therapy at the moment of blood sample withdrawal.

Standard thromboprophylaxis was administered in 200 patients (97.1%), while six patients (2.9%) received therapeutic doses of anticoagulation as they were on anticoagulant therapy prior to COVID-19 diagnosis, either with LWMH or direct oral anticoagulants (DOAC). Local protocol for thromboprophylaxis consisted in enoxaparin 40 mg/d or bemiparin 3500 UI/d.

With regard to the COVID-19 treatment, a total of 200 patients (98.1%) received hydroxychloroquine, the majority of them (91.7%) in combination with lopinavir/ritonavir. Corticosteroids were administered in 82 patients (39.8%) and 31 patients (15%) received tocilizumab. Less frequently used treatments included azithromycin (14.1%) and remdesivir (2.4%).

3.2 | Evaluation of coagulation parameters and inflammatory markers

The coagulation parameters assessment demonstrated normal median PT, INR, and APTT. However, D-dimer levels were particularly elevated (median 450 ng/mL; IQR 222.5-957.3) with 69.4% of patients above the normal range (0-250 ng/mL) and with the highest levels in patients on mechanical ventilation (median 1773 ng/mL; IQR 412-2812; $P = .03$). Mean fibrinogen levels were also above the upper limit (571.2 ± 187.7 mg/dL; normal range 200-400 mg/dL) which could be due to an acute phase response, as we found a statistically significant correlation between fibrinogen levels and other acute phase reactants such as C-reactive protein ($r = 0.67$; $P < .01$) and procalcitonin ($r = 0.33$; $P < .01$). There was no difference in fibrinogen levels based on oxygen requirements ($P = .20$).

Factor VIII levels showed an increasing trend (median 173.4%; IQR: 144.1-214.9; normal range 50%-200%), and factor IX was slightly above the upper limit of the normal range (median 142.8%; IQR 122.6-170.6; normal range 60%-140%). Factor VIII and IX were above the normal range in 32.9% and 56.1% of patients, respectively. The highest levels of factor VIII were observed in patients receiving high-flow oxygen therapy (median 203.8%; IQR 184.7-253.7) and mechanical ventilation (median 190.6%; IQR 167.1-234.5) ($P = .01$), and there were no statistically significant differences in factor IX levels regarding oxygen therapy ($P = .08$).

The remaining factors of the coagulation cascade were within normal limits (60%-140%). However, patients with higher oxygen requirements, especially those on mechanical ventilation, had lower levels of factor II (median 87.7%; IQR 81.7-102.6; $P = .03$), VII (median 67.1%; IQR 57.2-76.5; $P = .04$), X (median 81.3%; IQR 70.7-100.8; $P = .002$), and XII (median 97.1%; IQR 81.5-110.8; $P = .03$).

Regarding physiological coagulation inhibitors, free protein S levels were below the normal range (median 56.6%; IQR: 43.6-68.9; normal range 70%-140%) with 33% of patients showing levels below 50%. We observed the lowest levels of free protein S in patients receiving low-flow oxygen therapy (median 52.6%; IQR 42-63.4; $P < .001$). Protein C and antithrombin levels were within normal limits, and there was no difference in these parameters based on oxygen requirements.

Data collected from routine laboratory tests exposed that lymphocytes levels were decreased in our cohort (median $1 \times 10^3/\mu L$; IQR 0.4-0.8; normal range 1.3-$3.5 \times 10^3/\mu L$), with lower lymphocytes counts in patients on high-flow oxygen therapy (median $0.7 \times 10^3/\mu L$; IQR 0.4-1) and mechanical ventilation (median $0.9 \times 10^3/\mu L$; IQR 0.6-1.9; $P = .007$). Most acute phase reactants were elevated, with high levels of C-reactive protein (median 2.9 mg/dL; IQR 0.8-8.6; normal range 0-0.5 mg/dL), ferritin (median 710 μg/L; IQR 404-1389; normal range 5-204 μg/L), and interleukin 6 (median 34.4 pg/mL; IQR 7.6-90.8; normal range 0-4.3 pg/mL). These parameters were significantly more elevated in patients with higher oxygen requirements, with the highest levels of C-reactive protein (median 10.9 mg/dL; IQR 2.2-25.4; $P = .02$), ferritin (median 1998 μg/L; IQR 715.3-4009.3; $P < .001$), and interleukin 6 (median 148.3 pg/mL; IQR 107.8-1463.2; $P < .001$) been observed in patients on mechanical ventilation. All the other routine laboratory data analyzed were within normal levels (Table 1).

3.3 | Differences between survivors and non-survivors

Abnormal coagulation and other routine laboratory parameters were associated with poor prognosis. Notably, D-dimer levels were significantly higher in non-survivors (median 1472.5 vs 385 ng/mL; $P = .004$). Similarly, PT was more elevated in non-survivors (median 14 vs 12.7 s; $P < .001$). Significant differences were also observed in acute phase reactant levels between both groups (Table 1), and lymphopenia was associated with poor prognosis (median $0.6 \times 10^3/\mu L$ in non-survivors vs $1 \times 10^3/\mu L$ in survivors; $P = .007$).

Despite keeping within the normal range, non-survivors showed slightly lower levels of coagulation factors involved in the extrinsic (factor VII) and common (factor II and factor X) coagulation pathways, and this difference was statistically significant. We also found lower levels of protein C (median 107% vs 128%; $P = .001$) and antithrombin (median 86% vs 107%; $P = .008$) in the non-survivors (Figure 1).

Non-survivors had lower platelet count, and this was associated with poor prognosis (median $203 \times 10^3/\mu L$ vs $262 \times 10^3/\mu L$; $P = .013$). Thrombocytopenia was present in 44.4% of the non-survivors vs

8.5% of the survivors ($P < .001$). Criteria of overt disseminated intravascular coagulation (DIC) as defined according to the ISTH DIC score[11] were met in 22.2% of the non-survivors vs 3.7% of the survivors ($P = .001$).

3.4 | Patients with criteria of overt DIC

The 11 patients who met criteria of overt DIC were analyzed separately. The majority of these patients (54.6%) had high oxygen requirements and were either receiving oxygen via a high-flow device (9.1%) or mechanical ventilation (45.5%) ($P < .001$). Platelet counts were significantly lower in this subgroup (median 98 × 10³/μL vs

259 × 10³/μL; $P = .003$), D-dimer levels were higher (median 2812 vs 382 ng/mL; $P < .001$), and PT was prolonged (median 16.5 vs 12.7 s, $P < .001$). We found no statistical difference in fibrinogen levels (468 ± 193 vs 577 ± 186 mg/dL; $P = .06$). Levels of protein C (median 66% vs 127%; $P = .001$) and antithrombin (median 74% vs 107%; $P = .004$) were significantly decreased in patients with DIC, and we found lower levels of factor II (median 77.7% vs 102.3%; $P = .002$), X (median 45% vs 111.1%; $P < .001$) and XII (median 83.7% vs 117.3%; $P = .04$). Acute phase reactants showed also higher levels in patients with DIC, with C-reactive protein (median 10.8 vs 2.9 mg/dL; $P = .02$), procalcitonin (median 0.3 vs 0.06 μg/L; $P = .001$), and interleukin 6 (median 137.9 vs 33.6 pg/mL; $P = .002$) significantly more elevated in this subgroup.
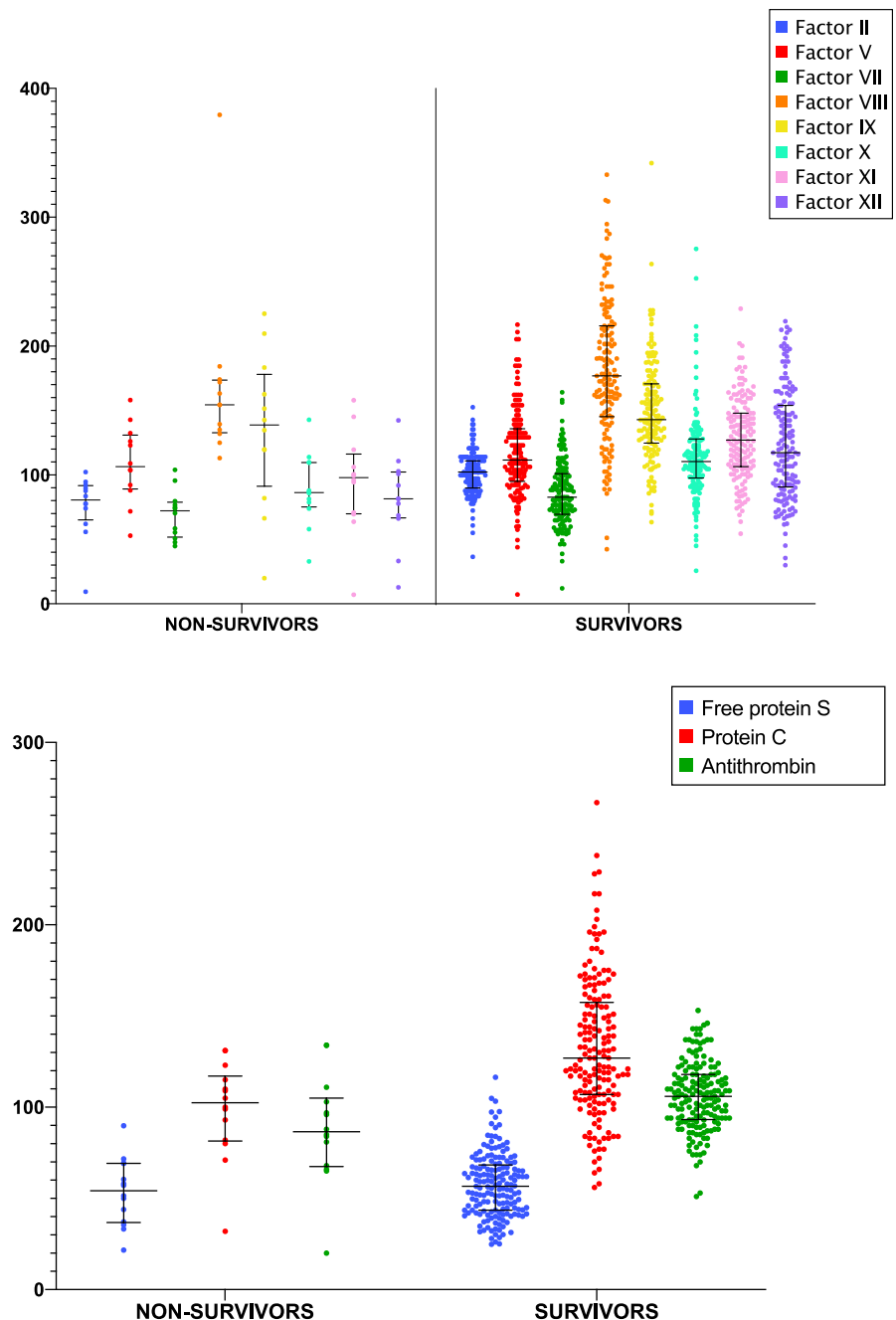
FIGURE 1 Comparison of coagulation factors and physiological inhibitory proteins between survivors and non-survivors. Lines represent median and Interquartile range. Factor II, VII and X levels were significantly lower in non-survivors ($P < .05$). Free protein S was found to be below the normal range in our sample. There was a correlation between lower levels of Protein C ($P = .001$) and antithrombin ($P = .008$) and poor prognosis [Colour figure can be viewed at wileyonlinelibrary.com]

3.5 | Comparison between ICU and non-ICU patients

Patients admitted in the ICU (12.6%) had more comorbidities (100% vs 75%; *P* = .004) than the rest of patients, and 15.4% of them met criteria of DIC (*P* = .015). Regarding the laboratory findings, these patients had lower platelet count (median 189 × 10^3/μL vs 259 x10^3/μL; *P* < .001), higher levels of factor V (median 135% vs 108%; *P* = .001), and lower of factor X (median 101% vs 111%; *P* = .003). Similarly, acute phase reactants were also more elevated, including C-reactive protein (median 3.9 vs 2.8 mg/dL; *P* = .009), ferritin (median 1038 vs 649 μg/L; *P* = .007), and interleukin 6 (median 138.3 vs 29.1 pg/mL; *P* < .001). In general, patients admitted in the ICU had a poorer prognosis, and 19.2% of them died (*P* = .04) (Table 2).

**TABLE 2** Differential characteristics of COVID-19-infected patients admitted in the ICU and in normal wards

Parameters	ICU (n = 26)	Non-ICU (n = 180)	*P* value
Demographics and clinical parameters			
Age (y)	59.9 ± 9.55	64.22 ± 13.84	.098
Sex (male/female)	18/8	113/67	.523
Comorbidities (yes/no)	26/0	135/45	.004[*]
DIC (yes/no)	4/22	7/173	.015[*]
Survivors (yes/no)	21/5	167/13	.043[*]
Thrombosis (yes/no)	5/21	13/167	.043*
Routine laboratory tests			
Hemoglobin (g/dL)	9.90 (8.85-12.99)	13.2 (12.23-14.30)	<.001[*]
Platelets (×10^3/μL)	189 (482.5-278.5)	262 (193.2-373.7)	<.001[*]
Leucocytes (×10^3/μL)	6.8 (4.5-10.4)	7.3 (5.4-9.8)	.561
Neutrophils (×10^3/μL)	5.6 (3.1-83)	5.5 (3.3-8.2)	.942
Lymphocytes (×10^3/μL)	0.7 (0.5-1.2)	1 (0.7-1.4)	.094
C-reactive protein (mg/dL)	3.2 (0.3-16.8)	2.9 (0.9-8.3)	.850
Procalcitonin (μg/L)	0.12 (0.05-0.32)	0.06 (0.03-0.12)	.009[*]
Ferritin (μg/L)	1031 (590-3267.5)	647.5 (378.2-1314)	.007[*]
Interleukin 6 (pg/mL)	136 (39.5-368.5)	30.8 (6.6-68.5)	<.001[*]
Coagulation parameters tests			
PT (s)	12.5 (12.1-13.4)	12.8 (12.1-13.7)	.367
INR	1.13 (1.08-1.22)	1.15 (1.09-1.23)	.374
APTT (s)	31.3 (26.4-33.4)	29.4 (26.8-32.3)	.251
Fibrinogen (mg/dL)	514.42 ± 212.7	579.4 ± 183.01	.218
D-dimer (ng/mL)	646 (218.5-1915.2)	411 (224-933.2)	.152
Free Protein S (%)	64.2 (45.3-76.6)	55.6 (43.5-67.1)	.162
Protein C (%)	121.5 (100.2-158.7)	127 (105-155)	.466
Antithrombin (%)	106.5 (90.5-120.2)	105 (92-116)	.873
Factor II (%)	99.7 (87.7-108)	102.3 (89.9-110.9)	.426
Factor V (%)	135.8 (108.9-184.7)	108.9 (92.2-132.5)	.001[*]
Factor VII (%)	88.3 (69.5-103.9)	81.7 67.9-99.3)	.906
Factor VIII (%)	190.6 (172.1-236.9)	170 (139.4-208.3)	.006[*]
Factor IX (%)	153.2 (128.2-164.5)	142.7 (121.7-170.6)	.520
Factor X (%)	98.4 (81.3-109.7)	111.1 (97.8-128)	.003[*]
Factor XI (%)	111.1 (102.4-139.9)	126.4 (104.7-150)	.275
Factor XII (%)	103.7 (81.5-115.1)	119.6 (87.3-153.9)	.186

Note: Data are presented as mean ± standard deviation or median (interquartile range) as appropriate.

Abbreviations: APTT, activated partial thromboplastin time; DIC, disseminate intravascular coagulopathy; INR, international normalized ratio; PT, prothrombin time.

*indicates statistically significant values.
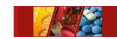
3.6 | Patients with arterial or venous thrombosis

Thrombotic events were observed in 23 patients during hospitalization, including venous thromboembolism in 18 patients (8.7%) and arterial thrombosis in 5 patients (2.4%). Venous thrombosis consisted in DVT in 10 patients (55.6%) and PE in 8 patients (44.4%), while arterial thrombosis manifested itself as myocardial infarction in 3 patients (60%) and acute lower limb ischemia in 2 patients (40%). Higher D-dimer levels were associated with higher risk of thrombotic events (1447.5 vs 380 ng/mL; $P < .001$). We found no statistically significant correlation between other abnormal coagulation parameters and higher risk of thrombosis (Table 3).

3.7 | Multivariate analysis

A multivariate analysis using a Cox proportional hazards regression model was performed to adjust for potential confounding factors

TABLE 3 Differential characteristics of COVID-19-infected patients with and without thrombosis

Parameters	Thrombosis (n = 23)	Non-thrombosis (n = 183)	P value
Demographics and clinical parameters			
Age (y)	64.1 ± 13.3	64.4 ± 13.5	.789
Sex (male/female)	17/6	114/69	.275
Comorbidities (yes/no)	22/1	139/44	.03[*]
DIC (yes/no)	3/20	8/175	.081
Survivors (yes/no)	20/3	168/15	.438
Routine laboratory tests			
Hemoglobin (g/dL)	13.15 (11.4-13.9)	13 (11.5-14.1)	.046[*]
Platelets (×10³/µL)	230.5 (191.5-362)	257 (176.5-366.5)	.224
Leucocytes (×10³/µL)	7.5 (5.5-10.3)	6.9 (5.4-9.3)	.183
Neutrophils (×10³/µL)	6 (3.9-8.2)	5.3 (3.4-7.6)	.211
Lymphocytes (×10³/µL)	0.9 (0.6-1.4)	1 (0.6-1.4)	.590
C-reactive protein (mg/dL)	2.2 (0.3-5.7)	3.3 (1.1-8.9)	.721
Procalcitonin (µg/L)	0.45 (0.03-0.6)	0.07 (0.03-0.1)	.233
Ferritin (µg/L)	615,5 (309.3-1435.5)	710 (412.5-1298)	.874
Interleukin 6 (pg/mL)	49.8 (16-254.5)	34.4 (8.1-77.1)	.105
Coagulation parameters tests			
PT (s)	12.7 (11.9-13.5)	12.7 (12-13.5)	.156
INR	1.11 (1.08-1.22)	1.15 (1.09-1.22)	.259
APTT (s)	31 (27-41.2)	29.6 (27.3-31.9)	.085
Fibrinogen (mg/dL)	513.6 ± 231.3	578.5 ± 180.9	.206
D-dimer (ng/mL)	1447.5 (547.7-2718.5)	380 (211.5-770.5)	<.001[*]
Free Protein S (%)	60.9 (46.3-69.4)	53.2 42.1-66.9)	.429
Protein C (%)	140 (122.3-163.5)	122 (105-157.5)	.419
Antithrombin (%)	98 (84.3-107.3)	108 (92.5-120)	.099
Factor II (%)	102.3 (88.3-107.3)	99.7 (87.7-110.9)	.6
Factor V (%)	130.8 (108.3-166.3)	108.9 (92.2-132.5)	.356
Factor VII (%)	82.25 (66.7-97.7)	81.7 (69.5-101)	.078
Factor VIII (%)	199.9 (162.3-231.6)	167.3 (130.5-208.3)	.054
Factor IX (%)	147.1 (110.7-188.8)	144.4 (126.1-169.8)	.984
Factor X (%)	104.7 (89.9-119.5)	112.5 (99-131.5)	.011[*]
Factor XI (%)	126.8 (103.8-150.5)	126.4 (105.7-147.3)	.611
Factor XII (%)	109.3 (94.5-134.9)	118.4 (87.3-154.7)	.189

Note: Data are presented as mean ± standard deviation or median (interquartile range) as appropriate.

Abbreviations: APTT, Activated partial thromboplastin time; DIC, disseminate intravascular coagulopathy; INR, International normalized ratio; PT, Prothrombin time.

*indicates statistically significant values.

such as sex, age, different timing between admission and blood sample collection and comorbidities, including those that could affect coagulation test results such as an active cancer, history of chronic liver disease, or abnormal liver function. This model revealed that age was an independent prognostic factor for survival (HR 1.32; 95% CI 1.09-1.62; *P* = .005) and there was a lack of association between D-dimer levels and poor prognosis (HR 0.99; 95% CI 0.99-1; *P* = .02). The remaining variables were not statistically significant. Meanwhile, the time-to-event Cox model to assess the risk of thrombosis confirmed that D-dimer was an independent predictor of the development of a thrombotic event (HR 1.99; 95% CI: 1.3-3.1; *P* = .002) and this was not interfered by other parameters such as sex, age, or comorbidities.

4 | DISCUSSION

Many reports have been published regarding coagulopathy in COVID-19-infected patients since the disease appeared.[5,6] Tang et al[7] first detected increased PT and higher levels of fibrinogen and D-dimer in the non-survivors group in a cohort of 183 patients. Similarly, our bivariate analysis proved that elevated D-dimer is associated with poor prognosis, with a fourfold increase among non-survivors. In the same line, we observed prolonged PT and lower platelet counts in non-survivors. However, the multivariate model revealed a lack of association between D-dimer levels and death (HR 1). This could imply that early reports on COVID-19 coagulopathy could have overrated the effect of D-dimer, as they did not perform a multivariate analysis, and the results could have been biased by potential cofounders. In consonance with this hypothesis, recent publications performing a regression test have shown results similar to ours, with values of HR close to 1 for D-dimer.[12,13]

The association of low platelet count, prolonged PT, and increased D-dimer observed in the first published reports is suggestive of DIC, even if the pattern is clearly different to DIC seen in other clinical situations such as sepsis or malignant tumors. In fact, a minority of patients meet criteria for DIC according to the ISTH score—5.3% in our cohort—in agreement with other data published.[14] This has led to the definition of a new concept coined as Pulmonary Intravascular Coagulopathy (PIC),[15] as distinct to DIC, which suggests that the diffuse bilateral pulmonary inflammation observed in COVID-19 may justify development of a pulmonary-specific vasculopathy and abnormalities in the coagulation parameters.

One of the novelties of our study is the exhaustive analysis of all the factors involved in the coagulation cascade, as well as the determination of physiological coagulation inhibitors with the purpose to asses whether a consumption coagulopathy is present in these patients. In the bivariate analysis, lower levels of factors II, VII, and X were associated with increased mortality and this could explain the prolongation of PT among non-survivors, even if these results did not reproduce when adjusting these variables for potential confounding parameters in the Cox regression. Factor VIII showed an increasing trend in the whole cohort, which could be interpreted

considering the inflammatory state and systemic cytokine storm present in these patients, as factor VIII has been reported to act as an acute phase reactant.[16] However, we found no correlation between factor VIII and mortality. Other authors have reported much higher levels of factor VIII in ICU patients[17]; these differences could be explained by the low rate of ICU patients in our cohort (12.6%).

Likewise, a mild protein S deficiency was found in our cohort. Protein S is a vitamin K-dependent protein and a physiological coagulation inhibitor. However, free protein S also binds to TAM—Tyro3, Axl, and Mer—receptors located on the surface of macrophages that are involved in homeostasis, inflammation, interaction with viruses, and pathophysiology of acute lung injury.[18-20]

Our study showed a moderate incidence of thrombotic events (11.1%) despite adequate thromboprophylaxis, in consonance with results recently published by Demelo-Rodriguez et al in a cohort of 156 patients from our hospital.[21] Further studies have reported a higher incidence of thrombosis,[22] but routine thromboprophylaxis was not standard of care. Other studies have found an increased incidence of thrombotic events, mostly in the ICU setting.[9,23] However, no correlation was found between abnormal coagulation parameters and higher risk of thrombotic events, beyond D-dimer levels (HR 1.99). One possible hypothesis for this discordance is the fact that patients with poorer prognosis may develop pulmonary microthrombi due to COVID-19-associated coagulopathy,[10] as demonstrated by autopsy findings,[24] which could have a significant impact in mortality. However, macroscopic thrombosis such as DVT and PE may not be as influenced by coagulation parameters alteration.

Some studies suggest that inflammation-induced endothelial cell injury could lead to an activation of the fibrinolytic system,[25,26] which may justify the elevated D-dimer levels in patients with severe COVID-19. Furthermore, it should be noted that a hyperfibrinolyic state and elevated levels of plasminogen activator inhibitor-1 (PAI-1) were observed in the SARS-CoV epidemics in 2002 [27] and similar alterations have been recently reported regarding SARS-CoV-2.[28] Therefore, a complete evaluation of the fibrinolytic system could provide further information about COVID-19-associated coagulopathy.

In addition, other possible underlying mechanisms of COVID-19 coagulopathy have been recently suggested. Elevated antiphospholipid antibodies have been observed in some patients[29,30] which could explain the endothelial damage that occurs in this disease and, thus, the coagulation abnormalities observed in these patients. The coagulation alterations observed in antiphospholipid syndrome are similar to those of COVID-19 coagulopathy, and they probably share some pathogenic mechanisms.[31] However, a recent study by Galeano-Valle et al[32] found no elevation of antiphospholipid antibodies in a series of 24 patients with COVID-19 pneumonia and venous thromboembolism. On the other hand, resemblance to thrombotic microangiopathy (TAM) has been suggested, with reportedly increased levels of von Willebrand factor (vWF) due to the vascular injury caused by SARS-CoV-2 endothelial infection. Furthermore, complement system activation might play a role in the endothelial damage caused by COVID-19, and the potential effect of anticomplement treatments is being studied.[33]

This study has some limitations. First, it was a single-centre cohort study with a restricted sample size, and 14.6% of patients remained hospitalized at the time of data collection. Second, coagulation parameter analysis was not performed on admission in all cases and this could bias our results, especially those concerning thrombotic risk. Third, information regarding bleeding events was not gathered. Fourth, certain conditions such as other active infections or medications could affect some of the coagulation tests results, especially those dependent on vitamin K, and that information was not gathered and could be a potential bias of our study. Finally, the study lacks a control arm and did not evaluate serum von Willebrand factor levels, which is emerging as a clinically relevant biomarker.

In conclusion, our findings confirm that severe COVID-19 infection is associated with coagulopathy that correlates with poor prognosis. However, it seems that COVID-19 coagulopathy is not a form of disseminated intravascular coagulation, as we did not demonstrate a consumption of coagulation factors in our cohort. Further studies to explore other possible mechanisms of this coagulopathy are required, including an exhaustive analysis of the fibrinolytic system and other coagulation parameters such as ADAMTS-13 or von Willebrand factor. Additionally, it would be interesting to perform future studies to assess whether any of the features observed in COVID-19 coagulopathy are predictive of hospitalization, in order to be able to act at an early stage of the disease.

CONFLICTS OF INTEREST
The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS
RM Martín-Rojas, G Pérez-Rus, and C Pascual contributed to the design of the study, data collection, statistical analysis, and writing and critical revision of the final manuscript. VE Delgado-Pinos contributed to the data collection. A Domingo-González, I Regalado-Artamendi, N Alba-Urdiales, S Monsalvo, G Rodríguez-Macías, M Ballesteros, and S Osorio-Prendes supplied the clinical data and participated in the clinical management of these patients. P Demelo-Rodríguez critically revised the manuscript. JL Díez-Martín coordinated the team. All authors contributed to literature review on the topic and critically reviewed the manuscript.

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