A mild deficiency of ADAMTS13 is associated with severity in COVID-19: comparison of the coagulation profile in critically and noncritically ill patients

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Early descriptions of COVID-19 associated coagulopathy identified it as a disseminated intravascular coagulation (DIC). However, recent studies have highlighted the potential role of endothelial cell injury in its pathogenesis, and other possible underlying mechanisms are being explored. This study aimed to analyse the coagulation parameters of critically and noncritically ill patients with COVID-19 bilateral pneumonia, determine if coagulation factors consumption occurs and explore other potential mechanisms of COVID-19 coagulopathy. Critically and noncritically ill patients with a diagnosis of COVID-19 bilateral pneumonia were recruited. For each patient, we performed basic coagulation tests, quantification of coagulation factors and physiological inhibitor proteins, an evaluation of the fibrinolytic system and determination of von Willebrand Factor (vWF) and ADAMTS13. Laboratory data were compared with clinical data and outcomes. The study involved 62 patients (31 ICU, 31 non-ICU). The coagulation parameters assessment demonstrated normal median prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time (APTT) in our cohort and all coagulation factors were within normal range. PAI-1 median levels were elevated (median 52.6 ng/ml; IQR 37.2–85.7), as well as vWF activity (median 216%; IQR 196–439) and antigen (median 174%; IQR 153.5–174.1). A mild reduction of ADAMTS13 was observed in critically ill patients and nonsurvivors. We demonstrated an inverse correlation between ADAMTS13 levels and inflammatory markers, D-dimer and SOFA score in our cohort. Elevated vWF and PAI-1 levels, and a mild reduction of ADAMTS13 in the most severe patients, suggest that COVID-19 coagulopathy is an endotheliopathy that has shared features with thrombotic microangiopathy. *Blood Coagul Fibrinolysis* 32:458–467

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Introduction

Since the first cases of SARS-CoV-2 infections were reported in Wuhan, China in late 2019 [1,2], this novel coronavirus has rapidly spread throughout the world, with more than 107 million cases and 2 million deaths as of February 13, 2021 [3]. During this period, several studies have been developed to better understand the pathogenesis and characteristics of this new disease. Among the most concerning hallmarks of COVID-19 infection is a coagulopathy that correlates with poor prognosis, as it was first stated by Tang *et al.* [4] in a single-centre study from China.

Early descriptions of this coagulopathy identified high levels of D-dimer and prolonged prothrombin time (PT) as typical features of COVID-19 infection [4,5]. This, together with the mild thrombocytopenia present in the most severe patients [6] and the evidence of microthrombi in postmortem studies [7,8], led to the assumption that COVID-19 associated coagulopathy could be a form of disseminated intravascular coagulation (DIC). Indeed, some reports and international guidelines recommended the use of the ISTH DIC score [9] to diagnose and classify COVID-19 coagulation abnormalities [10]. However, just a small group of patients meet criteria for DIC in the majority of published series, which suggests the underlying mechanism of COVID-19 coagulopathy is distinct from that of traditional systemic DIC [11,12].

Recently, some reports have highlighted the potential role of endothelial cells in the development of COVID-19 coagulopathy, proposing that we are rather facing an endotheliopathy than a consumption disorder [13,14]. Therefore, endothelial cells injury could be responsible for the coagulation alterations seen in these patients, and

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the prothrombotic manifestations often observed, such as venous, arterial or microvascular thrombosis. Direct viral infection of endothelial cells, collateral tissue damage or the release of inflammatory cytokines have been suggested as potential triggers of this new coagulopathy [15,16]. However, despite the efforts to understand COVID-19 coagulopathy, there is still a lot to explore to ascertain its underlying mechanism.

The aims of our study were to evaluate the coagulation parameters of critically and noncritically ill patients with COVID-19 bilateral pneumonia admitted to our hospital, assess if coagulation factors are consumed, identify potential prognostic biomarkers of this new disease and explore other possible underlying mechanisms of COVID-19 coagulopathy.

Materials and methods

We conducted a retrospective cohort study performed at Gregorio Marañon Hospital in Madrid. This study uses the method of a previously published study by our group, and the methods description partly reproduces its wording [12]. Adult patients with a diagnosis of COVID-19 bilateral pneumonia hospitalized in our centre were recruited, including those admitted to the ICU and to general wards. Patients were randomly selected from blood samples that arrived at our Hemostasis laboratory during April 2020. Once the patients were selected via this method, we requested extra citrated blood samples for the current research study.

We included patients with COVID-19 bilateral pneumonia aged 18 years or older hospitalized in our centre, regardless the time from admission. COVID-19 diagnosis was defined by positive PCR in nasopharyngeal swab or by radiologic and analytical findings highly suggestive of the disease. Patients receiving vitamin K antagonists (VKAs) less than 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. The need for consent was waived by the ethics committee due to the emergency status for the COVID-19 outbreak.

For each patient, we performed a complete analysis of the coagulation parameters. Demographic, clinical and routine laboratory data were obtained from electronic medical records using a standard data collection form. Routine laboratory data, according to our COVID-19 protocol, included cell blood count, biochemistry and acute phase reactants (C-reactive protein, procalcitonin, ferritin and interleukin-6). Severity of critically ill patients was assessed with the Sequential Organ Failure Assessment (SOFA) score, according to the new Sepsis-3 definition [17]. For each individual, clinical and routine laboratory data were collected at the time the haemostasis testing was performed. In addition, we retrospectively obtained routine laboratory data including blood cell counts and basic coagulation parameters on admission for a separate analysis.

Blood samples of selected patients were collected into 3.2% buffered sodium citrate solution-containing evacuated tubes and centrifuged for 20 min at 3000 rpm at room temperature. In total, we used four citrated blood samples per patient, of 3 ml each. All the coagulation parameters were analysed in the Hemostasis Laboratory of the Hematology department. Assays included PT, activated partial thromboplastin time (APTT), fibrinogen, D-dimer, quantification of coagulation factors and physiological inhibitor proteins (protein C, free protein S and antithrombin), evaluation of the fibrinolytic system (plasminogen, α_2-antiplasmin and plasminogen activator inhibitor-1), determination of von Willebrand Factor (vWF) activity and antigen and ADAMTS13. Basic coagulation parameters (PT, APTT and fibrinogen) and D-dimer assays were performed on freshly centrifuged samples. Unused plasma was aliquoted, nitrogen-frozen and stored at -80°C. The remaining coagulation tests were subsequently performed on these frozen samples. We did not use re-frozen samples for our study.

The PT, APTT and fibrinogen assays were determined on the ACL Top 700 analyser using HemosIL RecomplasTin 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 analyser (Instrumentation Laboratory, Bedford, Massachusetts, USA). 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. vWF Antigen was determined with its corresponding HemosIL reagent on the ACL Top 500. Plasminogen and α2-antiplasmin levels were determined with Plasminogen HemosIL and Plasmin Inhibitor HemosIL reagents respectively, both on the ACL Top 500. vWF activity, plasminogen activator inhibitor-1 and ADAMTS13 activity were determined with DG-EIA vWF Activity reagent (Diagnostic Grifols, Spain), ASSERACHOM PAI-1 reagent and DG-EIA ADAMTS13 Activity reagent (Diagnostic Grifols, Spain), respectively, all of them on the Triturus ELISA analyser.

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 nonnormal distribution. Normality was assessed using the Kolmogorov–Smirnov

Table 1 Demographics, clinical, laboratory and coagulation parameters of COVID-19 patients

Parameters	Normal values	Results ($n=62$)	Survivors ($n=51$)	Nonsurvivors ($n=11$)	P
Days until blood collection		10.5 (4–36.3)	8 (3–27)	36 (19–40)	0.015*
Demographics and clinical parameters					
Age (years)	–	61.8 ± 15.2	61.2 ± 15.4	64.7 ± 14.9	0.48
Sex (male/female)	–	43/19	35/16	8/3	0.78
Comorbidities (yes/no)	–	49/13	39/12	10/1	0.28
DIC (yes/no)	–	4/58	2/49	2/9	0.08
Routine laboratory tests					
Haemoglobin (g/dl)	12–16	11.6 ± 2.8	11.9 ± 0.4	10.1 ± 1.1	0.05
Platelets (x10^3/μl)	140–400	280.6 ± 144.5	303.4 ± 193.5	174.8 ± 391.8	0.006*
Leucocytes (x10^3/μl)	4–10	7.4 (5.4–10.3)	7.3 (5.4–9.3)	11.7 (4.9–15.8)	0.17
Neutrophils (x10^3/μl)	1.8–7.5	5.4 (3.6–8.1)	5.3 (3.5–7.5)	10.4 (4.7–14.1)	0.06
Lymphocytes (x10^3/μl)	1.3–3.5	1.1 ± 0.7	1.1 ± 0.9	0.7 ± 0.1	0.03*
C-reactive protein (mg/dl)	0–0.5	3.4 (0.6–8.6)	3 (0.5–8.2)	4.2 (0.7–9)	0.64
Procalcitonin (μg/l)	0–0.5	0.07 (0.04–0.12)	0.06 (0.03–0.1)	0.09 (0.05–0.36)	0.08
Ferritin (μg/l)	5–204	753 (382–1309)	602 (329.5–1180.2)	1178 (792–1634)	0.03*
Interleukin 6 (pg/ml)	0–4.3	30 (10–86.6)	17.8 (7.7–51.2)	129.5 (86.5–236.5)	0.001*
Coagulation parameters tests					
PT (s)	10.5–13.5	12.8 (12.1–13.7)	12.9 (12.1–13.7)	12.6 (11.3–15.2)	0.69
INR	0.8–1.2	1.15 (1.09–1.23)	1.15 (1.09–1.23)	1.14 (1.03–1.27)	0.76
APTT (s)	27–38	29.7 (27–33.1)	29.7 (27.3–32.8)	30.3 (25.7–44)	0.87
Fibrinogen (mg/dl)	200–400	565.7 ± 181.6	571.4 ± 23.7	539.5 ± 72.3	0.60
D-dimer (ng/ml)	0–250	549.5 (289–1071.2)	497 (248–958)	801 (407–1773)	0.24
Free Protein S (%)	70–140	60.6 ± 17.4	59.9 ± 2.2	64.1 ± 7.5	0.48
Protein C (%)	70–140	131.4 ± 40.1	131.8 ± 5.01	129.5 ± 18.9	0.87
Antithrombin (%)	70–140	101.8 ± 23.13	101.7 ± 2.7	102.7 ± 11.9	0.90
Factor II (%)	60–140	99.7 (87.2–108)	102.3 (89.9–108)	79.6 (64.7–94.6)	0.02*
Factor V (%)	60–140	120.8 ± 37.3	122.7 ± 5.4	112.2 ± 8.7	0.40
Factor VII (%)	60–140	81.3 ± 27.6	80.6 ± 3.6	84.4 ± 11.1	0.68
Factor VIII (%)	50–200	194.5 ± 71.9	182.3 ± 8.9	251.3 ± 24.7	0.003*
Factor IX (%)	60–140	148.1 ± 42.3	146.6 ± 5.3	155.3 ± 18.03	0.53
Factor X (%)	60–140	105.8 (87.5–124.6)	105.8 (88.7–124)	88.7 (81.3–129.7)	0.43
Factor XI (%)	60–140	122.7 ± 33.01	125.9 ± 3.8	108.1 ± 15.7	0.10
Factor XII (%)	60–140	111.2 ± 37.8	114.1 ± 4.9	98.03 ± 14.1	0.20
ADAMTS13 (%)	50–160	56.5 (44–80.8)	65 (47–88)	44 (36–56)	0.008*
vWF antigen (%)	62–175	216 (196–439)	211 (195.3–234)	511.1 (398–622.5)	0.001*
vWF activity (%)	58–163	174.1 (153.5–174.1)	171 (145.2–174.1)	174.1 (174.1–178.2)	0.028*
PAI-1 (ng/ml)	4–40	52.6 (37.2–85.7)	55.7 (38.6. 84.6)	35.1 (31.1–101.3)	0.23
α2-antiplasmin (%)	80–120	119.4 ± 12.7	119.8 ± 12.2	117 ± 15.1	0.53
Plasminogen (%)	75–135	101 ± 21.6	103.5 ± 20.4	89.1 ± 24.4	0.055

Data are presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for nonnormally distributed variables. APTT, activated partial thromboplastin time; DIC, disseminate intravascular coagulopathy; INR, International normalized ratio; PAI-1, plasminogen activator inhibitor 1; PT, prothrombin time; vWF, von Willebrand Factor. * indicates statistical significance ($p<0.05$).

test. The comparison between qualitative variables was analysed using Chi-squared test and Fisher's exact test. For quantitative variables, the *t*-test, the Mann–Whitney *U* and Kruskal–Wallis tests were performed depending on the normality of the variable. We used Spearman correlation and multiple linear regression to compare quantitative variables. Receiver operating characteristic (ROC) curves were used to determine the optimal threshold of ADAMTS13 to predict ICU admission and mortality. A multivariate analysis was performed using a logistic regression model. A two-tailed *P* value less than 0.05 was considered to be statistically significant for all analysis. Data were analysed using IBM SPSS Statistics for Mac (version 24. Armonk, New York, USA: IBM Corp) and GraphPad Prism (version 8.4.3; GraphPad Software, San Diego, California, USA).

Results

A total of 62 hospitalized patients with COVID-19 bilateral pneumonia were included in the final study cohort, and 50% of them were admitted to the ICU at the moment of blood sample withdrawal. Mean age of the patients was 61.8 (SD 15.2) years and 69.4% were male. Comorbidities were present in 49 patients (79%), being cardiovascular risk factors such as diabetes, hypertension and obesity the most frequently observed (87.8%) (Table 1).

In the ICU setting, the majority of patients (77.4%) were on mechanical ventilation at the time of blood sample collection, while high-flow oxygen therapy was used in the remaining 22.6%. However, patients admitted to general wards were either receiving low-flow oxygen therapy via nasal cannula (80.6%) or had no oxygen therapy requirement (19.4%) at the moment of blood sample withdrawal (Table 2).

All patients received standard thromboprophylaxis from admission, which consisted in enoxaparin 40 mg per day or bemiparin 3500 UI per day according to the local protocol. Despite this, 13 patients (21%) developed a thrombotic event during hospitalization in our cohort, including venous thromboembolism in 11 patients (17.7%) and arterial thrombosis in two patients (3.3%).

Table 2 Differential characteristics of COVID-19 patients admitted in the ICU and in normal wards

Parameters	ICU ($n=31$)	Non-ICU ($n=31$)	*P*
Days until blood collection	36 (19–42)	4 (3–9)	<0.001*
Demographics and clinical parameters			
Age (years)	59.5 ± 12.7	64.1 ± 17.3	0.237
Sex (male/female)	24/7	19/12	0.168
Comorbidities (yes/no)	25/6	24/7	0.755
DIC (yes/no)	4/27	0/31	0.039*
Survivors (yes/no)	21/10	30/1	0.003*
Thrombosis (yes/no)	9/22	4/27	0.12
Routine laboratory tests			
Haemoglobin (g/dl)	9.8 ± 2.5	13.4 ± 1.8	< 0.001*
Platelets (x10^3/μl)	249.8 ± 164.8	311.4 ± 115.5	0.093
Lymphocytes (x10^3/μl)	0.9 ± 0.7	1.2 ± 0.6	0.066
C-reactive protein (mg/dl)	5.9 (1.1–11.1)	2 (0.4–5.1)	0.046*
Procalcitonin (μg/l)	0.09 (0.05–0.31)	0.05 (0.03–0.08)	0.002*
IL-6 (pg/mL)	56 (16.8–145)	14.4 (6.6–50.8)	0.009*
Coagulation parameters tests			
PT (s)	12.9 (11.9–14)	12.7 (12.1–13.5)	0.714
APTT (sec)	30.8 (27.2–34.5)	29.1 (27.3–32.8)	0.554
Fibrinogen (mg/dl)	586.4 ± 198.2	545 ± 163.9	0.374
D-dimer (ng/ml)	801 (420–1541)	407 (213–580)	0.003*
Free Protein S (%)	62.7 ± 21.3	58.6 ± 12.7	0.357
Protein C (%)	126.9 ± 46.4	135.8 ± 33.1	0.392
Antithrombin (%)	98.9 ± 27.8	104.7 ± 17.9	0.331
Factor II (%)	99.7 (77.7–108)	102.3 (89.9–110.9)	0.472
Factor V (%)	131.9 ± 43.9	109.7 ± 25.3	0.017*
Factor VII (%)	79 ± 30	83.6 ± 25.3	0.521
Factor VIII (%)	234.4 ± 71.3	154.7 ± 46.6	< 0.001*
Factor IX (%)	154.2 ± 44.2	142.1 ± 40.2	0.264
Factor X (%)	92.9 (76.3–113.9)	115.4 (97.3–128)	0.006*
Factor XI (%)	118.6 ± 37.2	126.8 ± 28.3	0.333
Factor XII (%)	100.3 ± 32.9	122.2 ± 39.7	0.021*
ADAMTS13 (%)	45 (39–58)	80 (56–93)	< 0.001*
vWF antigen (%)	222.9 (191.9–489.3)	212.4 (198.1–237.9)	0.404
vWF activity (%)	174.1 (173–174.1)	166 (127–187)	0.110
PAI-1 (ng/ml)	38.4 (31.9–64.2)	67.9 (43.4–103)	0.003*
α2-antiplasmin (%)	92.2 ± 19.9	109 ± 20.2	0.002*
Plasminogen (%)	120.1 ± 13.8	118.7 ± 11.8	0.675

Data are presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for nonnormally distributed variables. APTT, activated partial thromboplastin time; DIC, disseminate intravascular coagulopathy; INR, international normalized ratio; PAI-1, plasminogen activator inhibitor 1; PT, prothrombin time; vWF, von Willebrand Factor. *indicates statistical significance ($p < 0.05$).

Conversely, 10 patients (16.1%) suffered haemorrhagic events, the majority of them (70%) being grade 1–2 according to the WHO bleeding scale. Forty percent of the patients who developed a haemorrhagic event were receiving full dose anticoagulation due to a prior thrombosis during hospitalization. There were no statistically significant differences between the incidence of thrombotic events in the ICU setting (69.2%) compared with patients in general wards (30.8%; $P = 0.12$). However, haemorrhagic events were more frequent in critically ill patients, with 90% of haemorrhages being documented in the ICU setting ($P = 0.007$) (Table 2).

At the time of writing, 46 patients (74.2%) had been discharged from hospital, five (8.1%) remained hospitalized and 11 (17.7%) had died. Median hospitalization time was 29 days (IQR 13–70). For critically ill patients, median stay in the ICU was 45 days (IQR 31–69).

Median days of hospitalization until the blood analysis was 10.5 days (IQR 4–36.3). This time was significantly higher ($P < 0.001$) in critically ill patients (median 36 days; IQR 19–42) compared with patients in general wards (median 4 days; IQR 3–9). As for nonsurvivors, median time until the blood analysis was 36 days (IQR 19–40) versus 8 days (IQR 3–27) for survivors ($P = 0.015$).

Data collected from routine laboratory tests showed an elevation of acute phase reactants in our cohort, with high levels of C-reactive protein (median 3.4 mg/dl; IQR 0.6–8.6; normal range 0–0.5 mg/dl), ferritin (median 753 μg/l; IQR 382–1309; normal range 5–204 μg/l) and interleukin 6 (median 30 pg/ml; IQR 10–86.6; normal range 0–4.3).

Regarding blood cell counts, mean platelets levels were within the normal range (280.6 ± 144.5 x 10^3/μl; normal range 140–400 x 10^3/μl), and only nine patients (14.5%) presented thrombocytopenia, of whom eight (88.9%) were admitted in the ICU ($P = 0.012$). Thrombocytopenia was also slightly more frequent among nonsurvivors (55.6 versus 44.4%; $P = 0.001$). Mean lymphocytes counts were decreased in our cohort (1.1 ± 0.7 x 10^3/μl; normal range 1.3–3–5 x 10^3/μl) and there was a correlation between lower lymphocytes levels and elevated inflammatory parameters, such as procalcitonin ($r = -0.28$; $P = 0.03$), ferritin ($r = -0.54$; $P < 0.001$) and interleukin 6 ($r = -0.51$; $P < 0.001$). Acute phase reactants were

further elevated in patients with poorer prognosis and, similarly, lower platelet count and a more profound lymphopenia were observed in critically ill patients (Table 2) and nonsurvivors (Table 1).

The evaluation of coagulation parameters demonstrated normal median PT, INR and APTT in our cohort. However, D-dimer levels were elevated (median 549.5 ng/ml; IQR 289–1071.2) with 79% of patients above the normal range (0–250 ng/ml). Likewise, mean fibrinogen levels were above the upper limit (565.7 ± 181.6 mg/dl; normal range 200–400 mg/dl). We found no statistically significant differences between these parameters and mortality (Table 1). However, there was a two-fold increase in D-dimer levels among patients in the ICU setting (median 801 versus 407 ng/ml; $P = 0.003$) (Table 2), with a positive correlation between D-dimer levels and SOFA score ($r = 0.43$; $P = 0.02$). D-dimer was also significantly higher in patients who developed a thromboembolic event during hospitalization (median 901 versus 459 ng/ml; $P = 0.03$).

Regarding coagulation factors, mean factor VIII levels showed an increasing trend (194.5 ± 71.9%; normal range 50–200%) and factor XI was slightly elevated (148.1 ± 42.3%; normal range 60–140%) in the whole cohort. Factor VIII was clearly elevated in nonsurvivors (mean 251.3 versus 182.3%; $P = 0.003$) and in critically ill patients (mean 234.4 versus 154.7%; $P < 0.001$). The remaining factors of the coagulation system were within normal limits (60–140%), and consumption was not observed (Table 1). Only four patients (6.5%) met criteria of overt DIC as defined according to the ISTH DIC score, all in the ICU setting. Free protein S levels were below the normal range (60.6 ± 17.4%; normal range 70–140%), with 24.2% of patients with levels below 50%. We found a statistically significant correlation between lower protein S levels and higher levels of acute phase reactants such as C-reactive protein ($r = -0.36$; $P = 0.004$) and procalcitonin ($r = -0.29$; $P = 0.02$). Protein C and antithrombin levels were within normal limits. We found no statistically significant differences between these physiological coagulation inhibitors levels and poor prognosis (Table 1).

Concerning the evaluation of the fibrinolytic system, plasminogen activator inhibitor-1 (PAI-1) median levels were elevated in our cohort (median 52.6 ng/ml; IQR 37.2–85.7; normal range 4–40 ng/ml), while α_2-antiplasmin and plasminogen levels were preserved. There were no statistically significant differences on these parameter levels based on mortality (Table 1). However, PAI-1 levels were paradoxically higher in non-ICU patients (median 67.9 versus 38.4 ng/ml; $P = 0.003$) (Table 2). We did not find any association between these fibrinolytic system parameters and the incidence of thrombosis in our cohort.

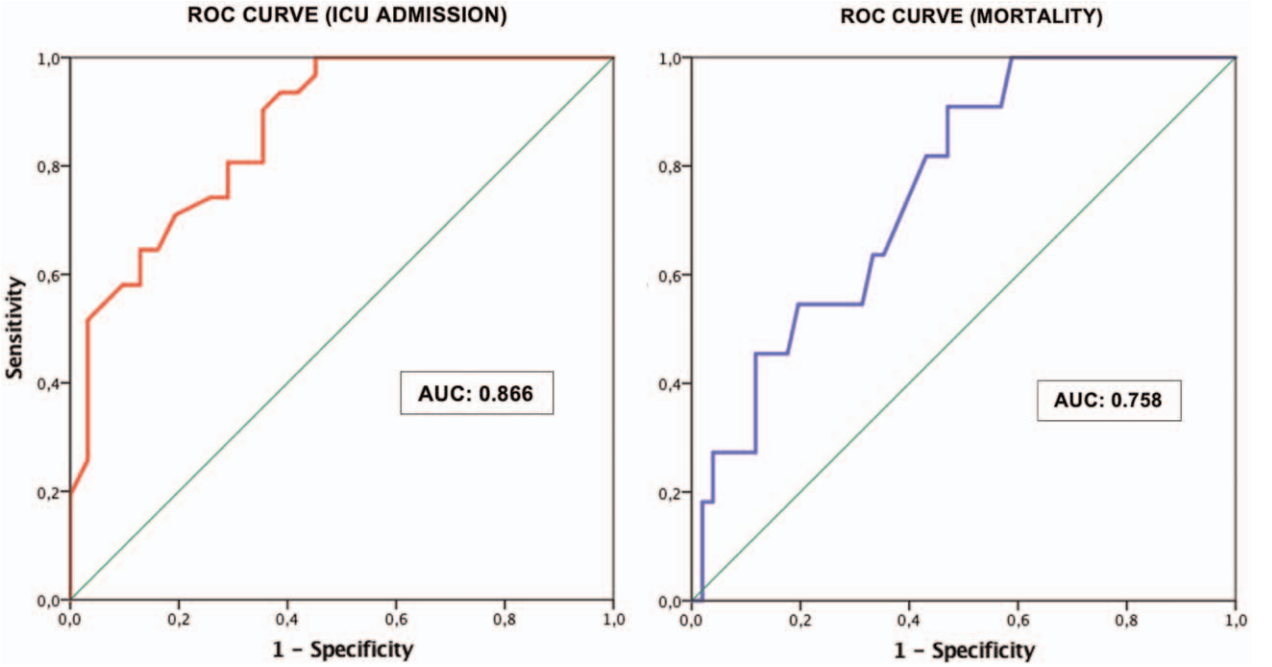
vWF antigen and activity levels were elevated in our sample, with a median level of 216% (IQR 196–439) and 174.1% (IQR 153.5–174.1) respectively. ADAMTS13 activity was generally preserved (median 56.5%; IQR 44–80.8; normal levels 50–160%) (Table 1). However, a mild reduction of ADAMTS13 activity was observed in critically ill patients (median 45 versus 80%; $P < 0.001$) (Table 2) and nonsurvivors (median 44 versus 65%; $P = 0.008$) (Table 1). The receiver operating characteristic (ROC) curve proved that ADAMTS13 showed an acceptable discriminative capacity to predict ICU admission (area under the curve 0.866; 95% CI 0.779–0.953; $P < 0.001$) and mortality (area under the curve 0.758; 95% CI 0.622–0.895; $P = 0.008$). The optimal cut-off value of ADAMTS13 activity to predict ICU admission was 60.5%, with a sensitivity and specificity of 80.6 and 71%, respectively. Alternatively, the optimal cut-off value to predict mortality was 56.5% (sensitivity 82%, specificity 57%) (Fig. 1).

We demonstrated an inverse correlation between ADAMTS13 activity and inflammatory markers such as ferritin ($r = -0.37$; $P = 0.006$), interleukin-6 ($r = -0.34$; $P = 0.01$), C-reactive protein ($r = -0.34$; $P = 0.006$) and Factor VIII ($r = -0.33$; $P = 0.004$), as well as with D-dimer levels ($r = -0.43$; $P < 0.001$), LDH ($r = -0.28$; $P = 0.03$) and SOFA score ($r = -0.38$; $P = 0.04$). Although not statistically significant, there was also a trend of inverse correlation between ADAMTS13 and vWF activity ($r = -0.24$; $P = 0.06$), and correlation between ADAMTS13 and vWF antigen did not reach statistical significance ($r = -0.13$; $P = 0.31$). Contrarily, ADAMTS13 values correlated directly with haemoglobin ($r = 0.43$; $P < 0.001$) and platelets count ($r = 0.35$; $P = 0.005$). Including all these parameters in a multiple linear regression model with backward stepwise, only SOFA score remained significantly associated with ADAMTS13 values (standardized β coefficient: -0.49; 95% CI -2.7 to -0.2; $P = 0.02$)

Stratifying the study population according to the abovementioned parameters values, ADAMTS13 progressively decreased from the lowest to the highest quartile of these variables, except for haemoglobin and platelets counts, where a positive correlation was observed (Fig. 2). We found no association between ADAMTS13 activity and the development of thrombotic events in our cohort (median 58 versus 56%; $P = 0.36$).

Due to the difference in median days of hospitalization until the blood samples for the present study were collected, a multivariate analysis using a Logistic Regression model adjusted for date of blood sample withdrawal was performed. This model revealed that lower ADAMTS13 values (OR 0.83; 95% CI 0.69–0.99; $P = 0.04$), lower levels of Factor X (OR 0.9; 95% CI 0.8–0.9; $P = 0.03$) and Factor XII (OR 0.96; 95% CI 0.93–0.99; $P = 0.01$) and increased C-reactive protein (OR 1.3; 95% CI 1.1–1–5; $P = 0.004$) and procalcitonin

Fig. 1



ROC curves for ICU admission (left) and mortality (right) prediction based on ADAMTS13 values. AUC, area under the curve; ROC, receiver operating characteristic.

(OR 9.9; 95% CI 1.5–69.6; $P = 0.02$) were independently associated with ICU admission in our cohort. However, lower levels of Factor II (OR 0.94; 95% CI 0.90–0.99; $P = 0.01$) and increased vWF antigen (OR 1.1; 95% CI 1.0–1.1; $P = 0.007$) were independently associated with mortality.

Finally, a secondary analysis was performed to elucidate whether blood cell counts and basic coagulation parameters on admission were able to predict prognosis in our cohort (Table 3). A moderate lymphopenia on admission was observed in our patients (median $0.7 \times 10^3/\mu l$; IQR 0.6–1.0), with the remaining median cell counts being within normal levels. Thrombocytopenia on admission was detected in nine patients (14.5%). A more profound lymphopenia on admission was observed among nonsurvivors (median $0.6 \times 10^3/\mu l$ versus $0.8 \times 10^3/\mu l$; $P = 0.04$) and patients admitted to the ICU (median $0.6 \times 10^3/\mu l$ versus $0.9 \times 10^3/\mu l$; $P = 0.01$). In addition, despite not reaching thrombocytopenia levels in most cases, lower platelet counts were found in nonsurvivors ($179 \times 10^3/\mu l$ versus $218 \times 10^3/\mu l$; $P = 0.03$) and patients who became critically ill during hospitalization ($188 \times 10^3/\mu l$ versus $233 \times 10^3/\mu l$; $P = 0.03$). Regarding coagulation parameters, median PT, INR and APTT on admission were within normal limits, with no differences observed based on mortality or ICU admission. However, mean fibrinogen (745 ± 174.8 mg/dl) and median D-dimer (390 ng/ml; IQR 233–979) levels were above the normal range, but none of these two parameters on admission showed statistically significant differences concerning mortality or ICU admission.

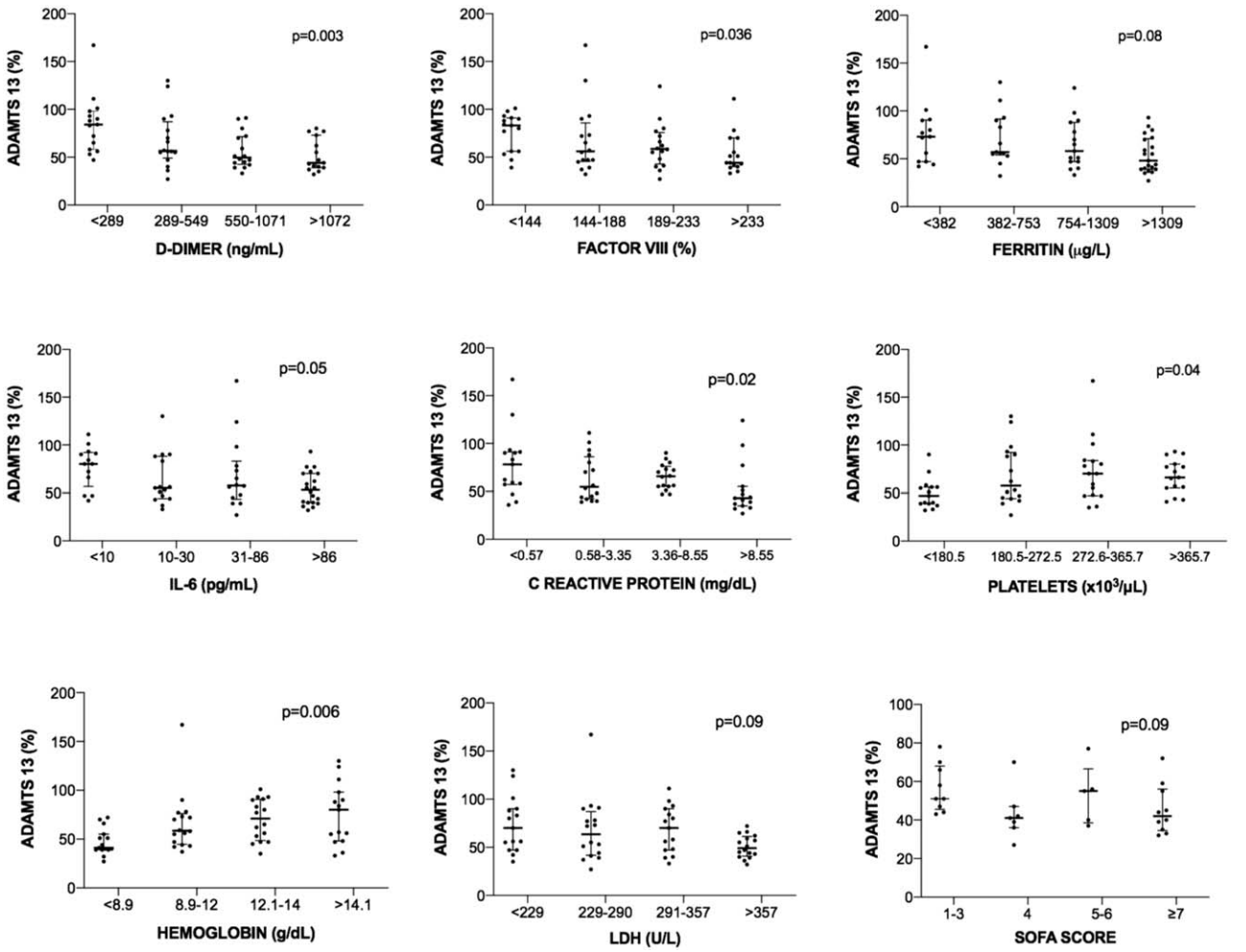
Discussion

An increasing number of scientific reports regarding coagulation abnormalities in COVID-19 have been published since the disease emerged in late 2019. In fact, COVID-19 associated coagulopathy has been subject of study over the last months, and an enormous effort has been made to decipher its underlying mechanism.

Early descriptions of this coagulopathy identified it as DIC [10]. However, more recent data suggest that COVID-19 coagulopathy differs from classical infection-induced DIC. In consonance with the latter idea, we demonstrated that coagulation factors were within normal levels in our cohort, and consumption was not observed. Moreover, in our study, only 6.5% of patients met criteria for DIC according to the ISTH score, in line with other published data [18,19,20]. These results are also in agreement with a recent publication by our group, in which we measured all coagulation factors in a larger cohort of 206 patients and we did not either observe consumption as seen in classical DIC [12].

However, a mild free protein S deficiency was found in our patients, with 24.2% of them showing levels below 50%. This reduction of protein S levels may be explained

Fig. 2



Lines represent median and interquartile range. *P* value was calculated using Kruskal–Wallis test. IL-6, interleukin 6; LDH, lactate dehydrogenase.

by an inflammatory mechanism rather than by consumption, as apart from being a vitamin K dependant physiological coagulation inhibitor, free protein S is also an activating ligand for the TAM family of receptors, that are located on the surface of macrophages and play a role in inflammation, virus interaction and pathophysiology of acute lung injury [21,22,23]. Indeed, we observed an inverse correlation between free protein S levels and

Table 3 Blood cell counts and basic coagulation parameters on admission

Parameters on admission	Total cohort ($n=62$)	Survivors ($n=51$)	Nonsurvivors ($n=11$)	*P*	ICU ($n=31$)	Non-ICU ($n=31$)	*P*
Platelets (x10^3/µl)	216 (166.7–279.7)	218 (175–286)	179 (97–223)	0.03*	188 (156–245)	233 (187–298)	0.03*
Leucocytes (x10^3/µl)	7.2 (5.3–10.6)	7.3 (5.4–10.5)	6.9 (3.2–11.4)	0.37	6.9 (4.6–11.4)	7.3 (5.4–8.8)	0.82
Neutrophils (x10^3/µl)	5.5 (3.6–9.5)	5.4 (3.9–9.8)	6.1 (1.7–9.4)	0.38	6.1 (3.4–10.2)	5.1 (3.8–7.8)	0.52
Lymphocytes (x10^3/µl)	0.7 (0.6–1.0)	0.8 (0.6–1.1)	0.6 (0.4–0.9)	0.04*	0.6 (0.4–0.9)	0.9 (0.7–1.2)	0.01*
PT (s)	13.5 (12.6–14.4)	13.5 (12.5–14.3)	13.4 (13–14.6)	0.85	13.4 (12.5–14.6)	13.5 (12.6–14.3)	0.93
INR	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.61	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.87
APTT (sec)	30.7 ± 3.8	30.4 ± 3.5	31.6 ± 4.8	0.36	30.7 ± 4.5	30.6 ± 2.9	0.98
Fibrinogen (mg/dl)	745 ± 174.8	733.8 ± 185.6	890 ± 101.4	0.25	739.9 ± 189.4	751.4 ± 161.9	0.79
D-dimer (ng/ml)	390 (233–979)	388 (234–1060)	435 (190–895)	0.93	435 (251–2486)	289 (230–659)	0.16

Data are presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for nonnormally distributed variables. APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time. *indicates statistical significance ($p<0.05$).

acute phase reactants which is consistent with the hypothesis that protein S is decreased by an inflammatory mechanism in these patients [24,25]. Apart from binding to macrophages, another feasible explanation for low free protein S in these patients could be an elevated C4BP (protein S binding protein). C4BP is also an acute phase reactant that is likely to be increased in COVID-19 and could lead to reduced levels of free protein S, while total protein S levels remain unchanged [26,27]. Finally, other mechanisms leading to protein S deficiency in COVID-19 patients have been recently proposed, such as the promiscuous cleavage of the papain-like protease (PL^{pro}) and protein S [28]. Considering all this, protein S deficiency in COVID-19 is likely to be multifactorial rather than due to a single mechanism, and further studies should be made on this aspect.

Furthermore, more recent reports have suggested that COVID-19 associated coagulopathy is an endotheliopathy that provokes increased vWF release, platelet activation and hypercoagulability [12,29]. Our data support that COVID-19 is accompanied by endothelial activation, as both vWF antigen and activity levels were elevated and significantly correlated with severity and mortality. Moreover, we also demonstrated high levels of PAI-1 in our cohort, as reported by Zuo *et al.* [30] who identified elevated levels of PAI-1 in a group of COVID-19 hospitalized patients, comparing with a cohort of healthy donors. This endorses the idea of endotheliopathy in COVID-19 patients, bearing in mind that endothelial cells are one of the main sources of PAI-1 [31], even if hepatocytes, adipocytes and megakaryocytes are also able to produce and secrete this protein [32].

In sepsis or inflammatory states, elevated vWF levels can be accompanied by a decreased ADAMTS13 activity [33]. Data regarding ADAMTS13 levels in COVID-19 infection is scarce. However, a few studies have recently reported a mild reduction of ADAMTS13 in these patients and its association with poorer prognosis [34–37].

One of the main findings of our study is a mild reduction of ADAMTS13 activity that correlates with severity and in-hospital mortality. A lack of severe ADAMTS13 deficiency in our patients excludes thrombotic thrombocytopenic purpura (TTP) and is more suggestive of secondary thrombotic microangiopathy (TMA) [38], which can be caused by viral infections [39–41]. The inverse correlation of ADAMTS13 levels and inflammatory markers found in our patients appears consistent with the hypothesis that the pro-inflammatory state of severe COVID-19 may inhibit the cleavage of vWF-ADAMTS13 or interfere with the proteolysis of ADAMTS13, leading to the development of microthrombi. These findings are in line with those observed in patients with sepsis and other viral infections in whom decreased ADAMTS13 levels are inversely correlated with vWF and severity [33,42]. Therefore, we suggest that the underlying mechanism of this alterations is the endothelial inflammation and damage, resulting in a mild reduction of ADAMTS13 levels. In consonance with all this, it is generally known that the endothelial damage triggers the coagulation cascade and releases fibrin degradation products (FDP), such as D-dimer. This FDP is routinely investigated in COVID-19 patients for its possible clinical impact on mortality, and it predicts ADAMTS13 levels in our cohort [43,44]. Furthermore, ADAMTS13 activity correlated inversely with SOFA score in our patients, which suggests that it may be a severity indicator in COVID-19 patients.

In addition, inflammatory response in TMA stimulates the activation of alternative complement pathway. Data from murine models highlight the activation of both lectin and alternative pathways in SARS-CoV-2 infection [45]. These findings could have a potential implication in the clinical management of COVID-19 severe patients, as plasma exchange and complement pathway blockers such as eculizumab might have a therapeutic potential [46,47,48]. However, further prospective studies should be performed to confirm our hypothesis.

This study has some limitations. First, it was a single-centre cohort study with a small number of patients, and 8.1% of them remained hospitalized at the time of data collection. Second, blood samples used for the analysis were not withdrawn on admission in all cases and this could bias our results. Third, we did not study the evolution of coagulation parameters at different moments during hospitalization. Fourth, some conditions such as concomitant infections or drugs could affect some of the coagulation tests results, and that information was not gathered and could be a potential bias of our study. Finally, some extra assays (such as C4BP, total protein S determination or vWF multimers) could have been performed to obtain additional information and explore other mechanisms of COVID-19 coagulopathy.

Conclusion

Our findings confirm that COVID-19 infection is associated with a coagulopathy that correlates with poor prognosis. However, coagulation factors and physiological inhibitor proteins levels have been preserved in our study, and we did not observe platelets or fibrinogen consumption, which leads us to assume that COVID-19 coagulopathy is not a form of DIC. Increased vWF and PAI-1 levels and decreased ADAMTS13 activity in our cohort could indicate that we are rather facing an endotheliopathy, which may have an overlapping pathophysiology with secondary TMA. Further prospective studies are required to better explore secondary TMA as the underlying mechanism of COVID-19 coagulopathy and to determine the potential effect of plasma exchange and complement pathway blockers as treatment options in the most severe patients.

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Conflicts of interest

The authors have no conflict of interest to declare.

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