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COVID-19-associated Coagulopathy Characterization using Rotational Thromboelastometry in a Prospective, Observational Cohort Study: The HemoCoV Study

Caracterização da Coagulopatia Associada ao COVID-19 usando Tromboelastometria Rotacional num Estudo Observacional de Coorte Prospetivo: Estudo HemoCov

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ABSTRACT

Introduction: COVID-19-associated coagulopathy includes systemic and endothelial inflammation with coagulation dysregulation related to immunothrombosis. The aim of this study was to characterize this complication of SARS-CoV-2 infection in patients with moderate to severe COVID-19.

Methods: An open-label, prospective observational study conducted in patients with COVID-19 moderate to severe acute respiratory failure admitted to an intensive care unit (ICU). Coagulation testing, including thromboelastometry, biochemical analysis and clinical variables, were collected at pre-specified time points during the 30 days of ICU stay.

Results: The study included 145 patients, 73.8% male, with a median age of 68 years (interquartile range - IQR 55 - 74). The most prevalent comorbidities were arterial hypertension (63.4%), obesity (44.1%) and diabetes (22.1%). Simplified acute physiology score II (SAPS II) was on average 43.5 (11 - 105) and sequential organ failure assessment (SOFA) at admission was 7.5 (0 - 14). During ICU stay, 66.9% of patients underwent invasive mechanical ventilation and 18.4% extracorporeal membrane oxygenation support; thrombotic and hemorrhagic events occurred in 22.1% and 15.1% of the patients respectively; anticoagulation with heparin was present in 99.2% of patients since early ICU stay. Death occurred in 35% of patients. Longitudinal studies revealed changes in almost all coagulation tests during the ICU stay. SOFA score, lymphocyte counts, some biochemical, inflammatory and coagulation parameters, including hypercoagulability and hypofibrinolysis seen in thromboelastometry, differed significantly ($p < 0.05$), between ICU admission and discharge. Hypercoagulability and hypofibrinolysis persisted throughout ICU hospitalization, showing higher incidence and severity in non-survivors.

Conclusion: COVID-19-associated coagulopathy is characterized by hypercoagulability and hypofibrinolysis from ICU admission, and persisted throughout the clinical course in severe COVID-19. These changes were more pronounced in patients with higher disease burden and in non-survivors.

Keywords: Blood Coagulation Disorders; COVID-19; Fibrinolysis; Thromboelastometry; Thrombosis

RESUMO

Introdução: A coagulopatia associada à COVID-19 inclui inflamação sistémica e endotelial com desregulação da coagulação relacionada com imunotrombose. O objetivo deste estudo foi caracterizar esta complicação da infecção por SARS-CoV-2 em doentes com infeção COVID-19 moderada a grave.

Métodos: Estudo prospetivo observacional *open-label* conduzido em doentes com insuficiência respiratória aguda COVID-19 moderada a grave admitidos numa unidade de cuidados intensivos (UCI). Testes da coagulação, incluindo tromboelastometria, testes de bioquímica e variáveis clínicas foram colhidos em pontos de análise predefinidos durante 30 dias de internamento na UCI.

Resultados: Foram incluídos 145 doentes, 73,8% homens, com uma mediana de idade de 68 anos (intervalo interquartílico – IIQ 55 - 74). As comorbilidades mais prevalentes foram hipertensão arterial (63,4%), obesidade (44,1%) e diabetes (22,1%). Na admissão, o *simplified acute physiology score II* (SAPS II) apresentou uma mediana de 43,5 (11 - 105) e o *sequential organ failure assessment* (SOFA) de 7,5 (0 - 14). Durante a estadia na UCI, 66,9% dos doentes foram submetidos a ventilação mecânica invasiva e 18,4% a suporte com *extracorporeal membrane oxygenation*; Eventos trombóticos e hemorrágicos ocorreram em 22,1% e 15,1% dos doentes respetivamente; anticoagulação com heparina esteve presente em 99,2% dos doentes desde precocemente durante a estadia na UCI. A morte ocorreu em 35% dos doentes. Estudos longitudinais revelaram alterações em quase todos os testes da coagulação durante a hospitalização na UCI. O SOFA *score*, a contagem de linfócitos, alguns parâmetros bioquímicos, inflamatórios e da coagulação, incluindo hipercoagulabilidade e hipofibrinólise observados na tromboelastometria, diferiram significativamente ($p < 0,05$), entre a admissão e a alta da UCI. A hipercoagulabilidade e a hipofibrinólise persistiram ao longo da hospitalização na ICU, mostrando maior incidência e gravidade nos doentes não sobreviventes.

Conclusão: A coagulopatia associada à COVID-19 é caracterizada por hipercoagulabilidade e hipofibrinólise desde a admissão na UCI, as quais persistiram durante o curso clínico na infeção COVID-19 grave. Estas alterações foram mais pronunciadas nos doentes com maior gravidade e nos não sobreviventes.

Palavras-chave: COVID-19; Fibrinólise; Perturbações da Coagulação Sanguínea; Tromboelastometria; Trombose

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INTRODUCTION

SARS-CoV-2 infection has been associated with a syndrome dominated by acute respiratory failure (ARF). However, the pathophysiology of this infection goes well beyond severe hypoxemia, its most feared manifestation. Immunological activation and coagulation dysregulation have been recognized as important mechanisms leading to thrombus-inflammation, thrombosis[1-3] and endothelitis.[1]

COVID-19 associated coagulopathy (CAC) is marked by profound hyperinflammation with liberation of inflammatory mediators,[4] endothelial dysfunction and injury, abnormal blood flow dynamics, platelet and coagulation factor activation, all of them leading to hypercoagulability and systemic fibrinolysis shutdown.[5,6] Moreover, real life evidence in severe COVID-19 shows hyperinflammation, T cell deficiencies and coagulation abnormalities associated with life-threatening organ dysfunction.[7] These complex chains of events induce a state of immunothrombosis leading to microvascular thrombosis.[1,4-6,8] The recognition of this prothrombotic state might explain why some laboratory parameters were viewed as potential surrogate markers of poor outcomes.[9-13] Dynamic cytokine storms and T cell lymphopenia are associated with COVID-19 severity as well,[7,14,15] making lymphocyte count another possible marker to identify patients at risk of developing severe COVID-19.[14]

Current International guidelines recommend prophylactic anticoagulation with heparin in all COVID-19 hospitalized patients, which should be tailored in selected patients.[16-21]

Assuming that coagulation abnormalities are paramount to define the outcome in COVID-19 patients, we designed a single-center study involving critically ill patients with SARS-CoV-2 infection and severe respiratory failure. We hypothesized that extensive CAC characterization may identify factors related to disease severity, contributing to a better understanding of COVID-19 pathophysiology.

METHODS

Study design and setting

The present study is part of a major research project named HemoCoV: an open-label, real-life, prospective non-interventional, cohort study, conducted by the Transfusion Medicine and the Intensive Care Departments of an Academic Tertiary Care Hospital Centre.

The HemoCoV study was approved by the Academic Hospital Centre Ethics Committee (reference number 295/20). STROBE recommendations for cohort studies were followed.[22]

Participants

Consecutive adult patients admitted to the Intensive Care Department (ICU) with a diagnosis of COVID-19-related acute respiratory failure (ARF) were evaluated for enrollment between August 20, 2020 and January 15, 2021.

Inclusion criteria were assessed and written informed consent was obtained in all patients.

The diagnosis of SARS-CoV-2 infection was confirmed by two positive polymerase chain reaction tests, in agreement with recommendations from national health authorities. Respiratory failure was defined as oxygen requirement administered by either high-flow nasal cannula, non-invasive ventilation or invasive mechanical ventilation (IMV), consistent with the World Health Organization (WHO) clinical progression scale definition of hospitalized severe disease.[23]

Inclusion and exclusion criteria

Inclusion criteria were patients aged 18 years or older with confirmed SARS-CoV-2 infection admitted to the ICU with moderate to severe hypoxemia (PaO_2/FiO_2 ratio below 200), irrespective of ventilatory status.

The exclusion criteria included pregnancy, previously diagnosed cognitive disorder (preventing informed consent), negative or SARS-CoV2 test not performed, absence of blood sample for thromboelastometry analysis within the first 24 hours of ICU admission, patients presenting with septic shock, severe liver failure, active cancer disease, history of congenital coagulation disorders, surgical procedures in the last four weeks, death before 24 hours of ICU admission, and withdrawal, refusal or inability to sign the informed consent form.

Sample size

In this study, 151 patients were included between August 2020 and January 2021. Six patients were withdrawn from the analysis for meeting the exclusion criteria. The reasons for exclusion were active cancer (n = 1), missing data (n = 3), death in the first 24 hours (n = 1) and SARS-CoV-2 infection not confirmed (n = 1).

Outcomes

The primary outcome was extensive CAC characterization at ICU admission and comparison with ICU discharge data.

The secondary outcomes were extensive CAC characterization at predefined time-points until day 30 of ICU stay or until ICU discharge and comparison of thromboelastometric parameters between survivors and non-survivors.

Intervention

Blood samples for AB0 blood type, complete blood count (CBC), thromboelastometry (ROTEM®), coagulation tests [D- dimer, prothrombin time–PT, activated partial thromboplastin time-aPTT, fibrinogen, factor (F) VIII, von Willebrand

factor (vWF): antigen (Ag) and ristocetin cofactor (RCo), antithrombin], interleukin-6 (IL-6), ferritin, C-reactive protein (CRP), procalcitonin (PCT), renal and liver function tests and blood gas (including ionized calcium-iCa^{2+}) were collected in the following time points: within 24 hours after ICU admission; immediately after implementing IMV or extracorporeal membrane oxygenation (ECMO); immediately after a diagnosis of a thrombotic or hemorrhagic event; every five-day interval along the initial 30 days of ICU admission; at ICU discharge. Blood samples were not reassessed if two events happened within four hours.

A clinically significant hemorrhagic event was defined as grade 2 or higher of the WHO bleeding score.[24] A clinically significant thrombotic event was defined as grade 2 or higher of the National Cancer Institute - Common Terminology Criteria for Adverse Events.[25] Glomerular Filtration Rate (GFR) was defined by the Chronic Kidney Disease Epidemiology Collaboration formula.[26,27] Computed tomographic angiography of the lung was performed in patients with clinical suspicion of lung microthrombosis or a major thromboembolic event. Doppler was done if there was clinical suspicion of a thrombotic event, and/or at ICU discharge. The hypercoagulability profile was defined by thromboelastometry parameters as clotting time (CT)-EXTEM < 45 seconds,[28] clot formation time (CFT)-EXTEM < 50 seconds,[29] maximum clot firmness (MCF)-EXTEM > 68 mm,[28,29] MCF-FIBTEM > 22 mm[28]; and hypofibrinolysis as lysis index at 60 minutes after CT (LI60)-EXTEM ≥ 97%,[28,29] lysis index at 30 minutes after CT (LI30) ≥ 97%, LI60 ≥ 97%, and maximum lysis (ML) < 5% in any thromboelastometry assay.

Additional assessments at specific time points (implementation of IMV and ECMO, diagnosis of thrombotic or hemorrhagic events) will be left for further evaluations.

Clinical data

Patient baseline characteristics were registered, including blood group, relevant comorbidities and previous antithrombotic therapy. Risk and prognostic scores were also evaluated [simplified acute physiology score II (SAPS II), sequential organ failure assessment (SOFA), sepsis-induced coagulopathy score SIC)[30] and disseminated intravascular coagulopathy score (DIC)].[31]

Clinical data were prospectively collected, including ventilatory mode, plateau pressure, driving pressure, $EtCO_2$/$PaCO_2$ ratio, and PaO_2/FiO_2 ratio. Anticoagulation therapy during ICU hospitalization was also registered. Hemorrhagic and thrombotic events were classified as complications, and triggered evaluation of coagulation parameters according to the national guidelines and as defined previously in this study, but will be left for further evaluation. Survival was defined by hospital discharge, either to the community or to another referring hospital. Mortality was defined as occurrence of death during the whole in-hospital stay, which includes ICU or subsequent ward admissions.

Laboratorial data

All laboratory assays were performed according to the standardized manufacturer protocols, comprising: CBC parameters (Coulter DX4900, Beckman-Coulter, California, USA); PT, aPTT, fibrinogen, FVIII, vWF:Ag, vWF:RCo, antithrombin (ACL TOP750, Werfen, Barcelona, Spain); D-dimer (ACL TOP750 CT5, Werfen, Barcelona, Spain); IL-6, ferritin, CRP, PCT, lactate dehydrogenase (LDH), creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, bilirubin, and troponin (Cobas 8000, Roche, Basel, Switzerland), and iCa^{2+} by blood gas analysis.

Thromboelastometry (ROTEM®)

Rotational thromboelastometry is a viscoelastic testing (VET) system, which evaluates the global coagulation process on whole blood.[28] VET allows for a global assessment of clot initiation (e.g., CT), clot strength or amplitude (amplitude at five minutes after CT-A5, amplitude at 10 minutes after CT-A10, MCF- e.g. fibrinogen and platelet contribution), and clot stability (e.g. fibrinolysis: LI30, LI60, ML).[32] In order to evaluate fibrinolysis, the established run time was 70 minutes to achieve ML and LI60. Four assays assessing extrinsic (EXTEM) and intrinsic (INTEM) clot activation, fibrinogen contribution to clot formation (FIBTEM), and heparin presence (HEPTEM) were done at the same time and at different time points of the previously described study analysis, using a ROTEM® Delta device (Werfen, Barcelona, Spain), and following the manufacturer`s instructions.

Statistical methods

Baseline clinical characteristics, as well as clinical and laboratory data, collected at the specified time points were compared between survivors and non-survivors.

Patient characteristics are presented as median, with the respective interquartile range (IQR) for the continuous variables, according to the distribution underlying the data, and as number (n) and percentage (%) for the categorical variables. The normality underlying the data was evaluated using the Shapiro-Wilk test.

Statistical comparisons between two independent groups were performed with the Mann–Whitney U test for continuous variables and with the χ^2 test for categorical variables or Fisher's exact test when applicable. Regarding paired data, statistical comparisons were performed using the Wilcoxon test.

All the results with a p-value < 0.05 were considered significant. The statistical analysis was performed using the software R Studio version 4.1.2. In case of missing data for

a specific variable, these patients were not included in the analysis including this same variable. No imputation was performed.

RESULTS

Sample characteristics

A total of 151 consecutive patients were included. Of these, six patients were excluded. The remaining 145 patients were eligible and were included in the analysis. The patient's main characteristics at ICU admission and during ICU hospitalization are described in Tables 1 and 2, respectively.

Patients included in our study were predominantly white and male (86.9% and 73.8%, respectively) with a median age of 64 years (IQR 55 - 74) (Table 1). Arterial hypertension was the most prevalent comorbidity (63.4%). Previous thrombosis was documented in six patients. At ICU admission, 95 patients were already under anticoagulant therapy, mainly prophylactic low molecular weight heparin (LMWH).

During ICU hospitalization (Table 2), 66.9% required IMV, and 18.4% were treated with ECMO support. A thrombotic or hemorrhagic event occurred in 22.1% and 15.1% of patients, respectively. From early on during the ICU stay, 99.2% of patients received anticoagulation, mostly with prophylactic and intermediate doses of LMWH. Overall, a mortality rate of 35% was observed.

Clinical characteristics

Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148) shows the evolution of different laboratory parameters plus clinical scores at different time points of the analysis during the 30 days of ICU stay. The main parameters with a significant *p*-value ($p < 0.05$) evaluated

Table 1 – Patient characteristics at Intensive Care Unit admission

Parameter	n (%)	IQR
Total number of patients	145	
White	126 (86.9)	
Male	107 (73.8)	
Age (median) (years)	64	55 - 74
Weight (median) (kg)	82	73 - 96
Symptom-to-hospital admission (median)(days)	7	5 - 10
Symptom-to-ICU admission (median)(days)	9	7 - 12
Blood groups:		
Group O:	52 (35.9)	
Group non-O:	87 (60)	Non-O: A:63; B:17;AB:7
Unclassified:	6 (4.1)	
Comorbidities:		
Arterial hypertension	92 (63.4)	
Obesity	64 (44.1)	
Diabetes	32 (22.1)	
Previous lung disease	32 (22.1)	
Heart disease	31 (21.4)	
Chronic kidney disease	18 (12.4)	
Previous thrombosis	6 (4.10)	
Anticoagulation at ICU admission	95 (65.5)	LMWH:87, UFH:7, EDX:1, NA:50
Antiplatelet therapy at ICU admission	19 (13.1)	AAS:15; Clop:3; AAS+Clop:1
Clinical Scores	**Median**	**Variations**
SAPS II	43.5	11 - 105
SOFA	7.5	0 - 14
SIC	3	2 - 5
DIC	1	0 - 6

AAS: acetylsalicylic acid; DIC: disseminated intravascular coagulopathy; EDX: edoxaban; Clop: clopidogrel; ICU: Intensive care unit; IQR: Interquartile range; LMWH: Low molecular weight heparin; NA: Not available; SAPS II: simplified acute physiology score II; SIC: Sepsis-induced coagulopathy; SOFA: Sequential organ failure assessment; UFH: Unfractionated heparin.

Table 2 – Patient characteristics during Intensive Care Unit hospitalization

Parameter	n (%)	IQR
ICU length-of-stay (median) (days)	14	5 - 27
Hospital length-of-stay (median) (days)	26	14.25 - 43.75
Anticoagulation during ICU hospitalization:		
Day 5 (n = 125)	124 (99.2%)	LMWH:108; UFH:15; Biva:1
Day 30 (n = 35)	33 (94.3%)	LMWH:25; UFH:6; Biva:2
ICU Discharge (n = 145)	139 (95.99)	LMWH:131; UFH:9
Invasive mechanical ventilation	97 (66.9)	
ECMO support	26 (18.4)	
Thrombotic events	32 (22.1)	
Hemorrhagic events	22 (15.1)	
Mortality rate:		
Global	51 (35.0)	
At ICU	43 (29.7)	
At hospital (after ICU discharge)	8 (5.50)	

Biva: bivalirudin; ECMO: extracorporeal membrane oxygenation; ICU: Intensive Care Unit; IQR: interquartile range; LMWH: low molecular weight heparin; UFH: unfractionated heparin.

at admission *versus* at discharge are shown [full data is displayed in the supporting information (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15151)].

Lymphocyte and other blood counts, biochemical (LDH, PCT, iCa^{2+}, creatinine, GFR, AST, ALT, albumin, troponin), inflammatory (CRP, IL-6, ferritin), and coagulation (aPTT, fibrinogen, FVIII, antithrombin) parameters, as well as SOFA score, differed significantly ($p < 0.05$) between day of admission and of discharge (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148). The same was seen concerning thromboelastometric findings of hypercoagulability (EXTEM-CFT; FIBTEM-A5, A10, MCF) and of hypofibrinolysis (EXTEM-LI60, ML; INTEM and HEPTEM-LI30, LI60, ML).

On the other hand, no statistically significant differences were seen for the other coagulation parameters (D-dimer, international normalized ratio (INR), vWF:Ag, and vWF:RCo), and for the SIC and DIC scores (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/

Table 3 – Thromboelastometry parameters: hypercoagulability and hypofibrinolysis in survivors versus non-survivors at Intensive Care Unit (ICU) admission and day 30 of ICU hospitalization (percentage of patients)

Thromboelastometry	ICU Admission-DAY 0	Survivors (n = 94)	Non-survivors (n = 51)
Hypercoagulability	CFT-EXTEM < 50 sec.	37.2%	29.4%
	MCF-EXTEM > 68mm	61.7%	56.9%
	MCF-FIBTEM > 22mm	98.9%	96.0%
Hypofibrinolysis	LI60-EXTEM ≥ 97%	26.9%	42.9%
	ML-EXTEM < 5%	9.5%	23.5%
	LI30-INTEM ≥ 97%	95.7%	98.0%
	Day 30 at ICU	**Survivors** (n = 94)	**Non-survivors** (n = 51)
Hypercoagulability	CFT-EXTEM < 50 sec.	28.5%	28.5%
	MCF-EXTEM > 68mm	50.0%	50.0%
	MCF-FIBTEM > 22mm	57.1%	78.6%
Hypofibrinolysis	LI60-EXTEM ≥ 97%	50.0%	78.6%
	ML-EXTEM < 5%	37.5%	42.9%
	LI30-INTEM ≥ 97%	100%	100%

CFT: clot formation time; ICU: Intensive Care Unit; LI30: Lysis index at 30 minutes after CT; LI60: Lysis index at 60 minutes after CT; MCF: maximum clot firmness; ML: maximum lysis; sec: seconds; EXTEM/FIBTEM/INTEM: thromboelastometry assays.
Percentage of survivor versus non-survivor patients showing the described thromboelastometry parameters at Intensive care unit (ICU) admission and on day 30 of ICU hospitalization.

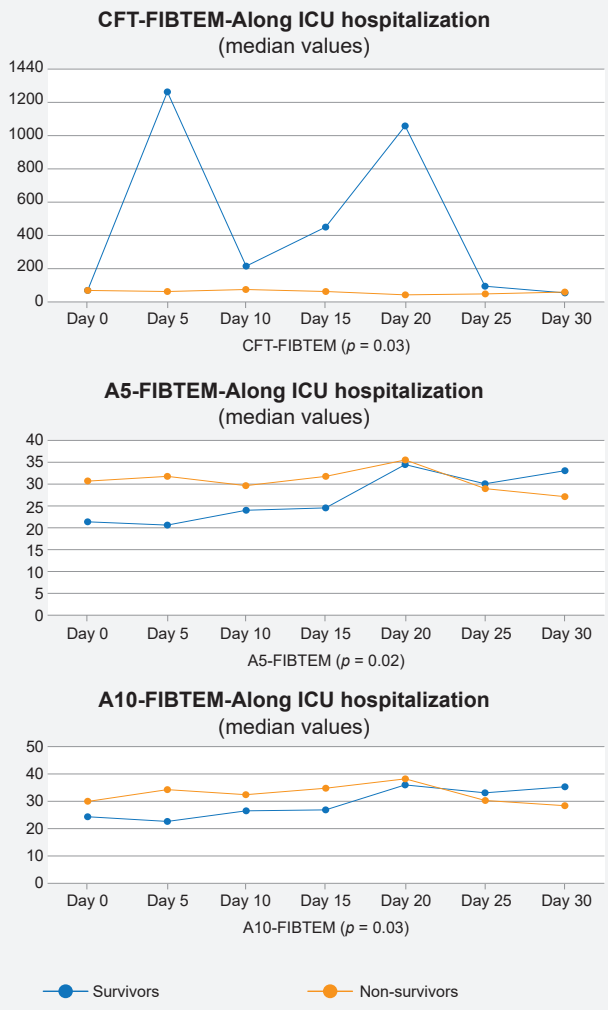

Figure 1 – Hypercoagulability along 30 days of Intensive Care Unit hospitalization: comparison between survivors (n = 94) and non-survivors (n = 51) (median values).
A5: amplitude at 5 minutes after CT; A10: amplitude at 10 minutes after CT; CT: clotting time; CFT: clot formation time; ICU: Intensive Care Unit. FIBTEM thromboelastometry assay.
[*p*-value from Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148)].

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During ICU hospitalization (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148), a slightly higher number of lymphocytes from day ten (D10) was observed, but median values over $1x10^6$/L were only seen at D30 and at ICU discharge. A slight decrease in the level of inflammatory parameters (CRP, ferritin) was observed, although elevated levels were still observed at D30 and ICU discharge. However, normal median values of IL-6 were seen consistently from D15.

During the ICU stay, hypercoagulability was expressed through consistent persistently high levels of FVIII, vWF:Ag, vWF:RCo, and by thromboelastometry parameters (FIBTEM: A5, A10, MCF). Hypofibrinolysis expressed by thromboelastometry parameters (mainly by median values of INTEM/HEPTEM - LI30, LI60, ML), was also observed throughout ICU hospitalization, mostly until D30 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148). For all the other evaluated parameters, there were no significant differences between the different time points (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15151).

Comparing data from survivors and non-survivors, at ICU admission and on D30 of ICU stay (Table 3), patients presented with hypercoagulability and hypofibrinolysis, as defined elsewhere,[28,29] with greater incidence and severity in non-survivors. Major and significant differences between survivors and non-survivors were observed regarding the presence and severity of hypofibrinolyis, being more pronounced in non-survivors (Table 3, Figs. 1 and 2).

Higher hypercoagulability (FIBTEM: CFT, A5 and A10) (Fig. 1) and greater hypofibrinolysis in all assays (EXTEM/HEPTEM: LI60 and ML; INTEM: LI30, LI60 and ML) (Fig. 2) were observed in non-survivors compared with survivors along the 30 days of ICU hospitalization.

Most patients received prophylactic and intermediate doses of LMWH. No differences in the hypercoagulability and hypofibrinolysis profiles were observed in thromboelastometry between patients receiving prophylactic and intermediate heparin doses.

DISCUSSION

HemoCoV is a real world, prospective and non-interventional study that evaluated COVID-19-associated coagulopathy (CAC) and its implications in the clinical course of SARS-CoV-2 infection along 30 days of ICU hospitalization. One hundred and forty-five consecutive patients with COVID-19 moderate to severe acute respiratory failure were assessed for extensive evaluation of coagulation parameters, using conventional tests and thromboelastometry. Despite anticoagulation treatment, thromboelastometry still revealed significant coagulation abnormalities expressed by hypercoagulability and hypofibrinolysis, since ICU admission (initial phases of disease) and persisting throughout the clinical course. Both changes were more pronounced in patients with higher disease severity and in non-survivors. More prevalent and severe hypofibrinolysis was observed in non-survivors. This may reflect a significant role of hypofibrinolysis in the pathophysiology of severe COVID-19.

Similar results have been previously described,[33-36] suggesting that thromboelastometry parameters may be useful to distinguish coagulation patterns between patients with non-severe and severe COVID-19.[35] Some authors additionally suggest that this phenotypic characterization may be useful for personalized therapeutic intervention.[35] None of our patients had CT-EXTEM under 45 seconds, often

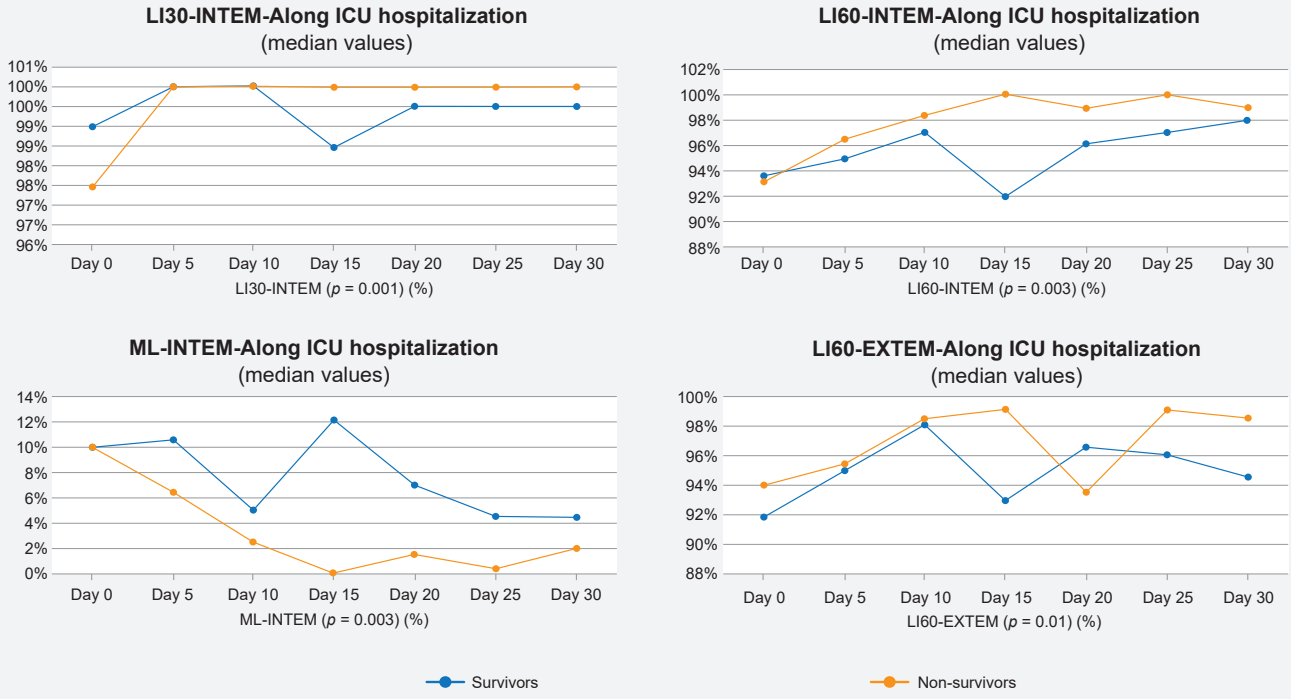

Figure 2 – Hypofibrinolysis along 30 days of Intensive Care Unit hospitalization: comparison between survivors (n = 94) and non-survivors (n = 51) (median values).
CT: clotting time; ICU: Intensive Care Unit; MCF: maximum clot firmness; LI30: Lysis index at 30 minutes after CT; LI60: Lysis index at 60 minutes after CT; ML: maximum lysis; Maximum lysis (ML): is the reduction of clot firmness after the MCF. INTEM/EXTEM thromboelastometry assays.
[*p*-value from Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148)].

linked to hypercoagulability,[28] as previously described in other studies.[35] The higher burden of fibrinogen and other coagulation factors (FVIII, vWF) along the clinical course of COVID-19, was observed in our study also by the elevated levels of CT-FIBTEM and no significant differences between ICU admission and discharge. To stress the clinical importance of hypercoagulability, some authors[35,37] propose a cut-off for MCF-FIBTEM over 27mm to differentiate between severe and non-severe forms of COVID-19 disease, with high accuracy.[35] In our patients the median values of MCF-FIBTEM were above the normal range from ICU admission to discharge, although showing a significant difference (p = 0.02). Concerning fibrinolysis, there have been suggestions of a functional shutdown in patients with severe COVID-19.[38-40] This description matches our findings, which revealed decreased fibrinolysis, suggesting a prothrombotic condition and thus compromising vascular permeability. This finding is not always reproducible,[35] which highlights the complex interactions of the coagulation system.

D-dimer levels in our population did not differ significantly between time point analyses, despite previous suggestions of it being a reliable predictor of disease severity or a prognostic marker for in-hospital mortality.[41] Conversely, as others described,[42] fibrinogen levels were significantly different from ICU admission to discharge (p < 0.001), and high prevalence of increased fibrinogen levels (> 7 g/L) at admission[43] were observed. Hence, a careful assessment of fibrinogen is required for stratifying CAC, which may have been overlooked and may need to be revisited.[42]

As previously described in the recent literature,[44] severe lymphopenia was identified in our study with a statistically significant difference in lymphocyte count between ICU admission and discharge (p < 0.001), reflecting the severity of this viral disease.

To the best of our knowledge, our study is one of the first where different coagulation tests (standard coagulation and viscoelastic tests) as well as biochemical and clinical parameters were prospectively and extensively analyzed in COVID-19 patients during 30 days of ICU hospitalization. This allowed us to analyze CAC with a holistic view, identifying coagulation abnormalities that may have a clinical impact on future evaluation and treatment of these patients. One of the strengths of our study is the longitudinal temporal characterization of coagulation abnormalities. Almost all coagulation tests evaluated differed from the initial to the final stages of the disease. The changes documented in our

study strongly suggest that pathophysiological mechanisms involved in COVID-19 may be related to a hyperthrombotic profile along with hypofibrinolysis. This could be extremely useful in clinical practice, namely for defining distinctive therapeutic interventions, such as anticoagulation implementation and dosage, and for stratification of the patient's individual risk in subsequent studies. Moreover, and by being a single center study, it allowed us to ensure similar established interventions and homogeneous therapeutic and intensive care interventions.

Our study has several limitations. Firstly, it is a single-center, open-label, and non-randomized study, with a specific cohort that might not be representative of other populations. As genetic factors are known to affect the coagulation system, our findings should be reproduced and evaluated in other settings and other genetic backgrounds. Additionally, it should be recognized that this study took place during the SARS-CoV-2 delta variant pandemic wave, and hence there is no irrevocable evidence that similar results would occur with other coronavirus variants.

CONCLUSION

Our study found that hypercoagulability and hypofibrinolysis, as assessed by rotational thromboelastometry, are present from ICU admission and persist throughout the clinical course of severe COVID-19 patients, despite receiving anticoagulation treatment. This profile was more pronounced in patients with higher disease severity. Hypofibrinolysis was more prevalent and more severe in non-survivors, which may reflect its significant role in the pathophysiology of severe COVID-19. Better characterization of COVID-19-associated coagulopathy might help identify severe COVID-19 disease and poor outcomes.

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AWARDS AND PREVIOUS PRESENTATIONS

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AUTHOR CONTRIBUTIONS

AR: Concept and design of the study, data collection, analysis and interpretation of data, critical writing, revision and approval of the final version.

TDD, GNJ: Analysis and interpretation of data, critical writing, revision and approval of the final version.

AG, ARR, CJC: Data collection, analysis and interpretation of data, revision and approval of the final version.

CLP: Data collection, revision and approval of the final version.

DC: Data collection, analysis and interpretation of data, revision and approval of the final version.

AB: Revision and approval of the final version.

JMR: Analysis and interpretation of data, revision and approval of the final version.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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