Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Thrombosis Research 196 (2020) 359–366

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Therapeutic versus prophylactic anticoagulation for severe COVID-19: A randomized phase II clinical trial (HESACOVID)

Anna Cristina Bertoldi Lemos[a], Douglas Alexandre do Espírito Santo[a], Maísa Cabetti Salvetti[b], Renato Noffs Gilio[b], Lucas Barbosa Agra[a], Antonio Pazin-Filho[a], Carlos Henrique Miranda[a,*]

[a] Division of Emergency Medicine, Department of Internal Medicine, Ribeirão Preto School of Medicine, São Paulo University, Ribeirão Preto, SP, Brazil
[b] Hospital Estadual de Américo Brasiliense, Ribeirão Preto School of Medicine, São Paulo University, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Keywords:
COVID-19
Mechanical ventilation
Anticoagulant treatment
D-dimer
Coagulopathy

ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) causes a hypercoagulable state. Several autopsy studies have found microthrombi in pulmonary circulation.

Methods: In this randomized, open-label, phase II study, we randomized COVID-19 patients requiring mechanical ventilation to receive either therapeutic enoxaparin or the standard anticoagulant thromboprophylaxis. We evaluated the gas exchange over time through the ratio of partial pressure of arterial oxygen (PaO2) to the fraction of inspired oxygen (FiO2) at baseline, 7, and 14 days after randomization, the time until successful liberation from mechanical ventilation, and the ventilator-free days.

Results: Ten patients were assigned to the therapeutic enoxaparin and ten patients to prophylactic anticoagulation. There was a statistically significant increase in the PaO2/FiO2 ratio over time in the therapeutic group (163 [95% confidence interval – CI 133–193] at baseline, 209 [95% CI 171–247] after 7 days, and 261 [95% CI 230–293] after 14 days), p = 0.0004. In contrast, we did not observe this improvement over time in the prophylactic group (184 [95% CI 146–222] at baseline, 168 [95% CI 142–195] after 7 days, and 195 [95% CI 128–262] after 14 days), p = 0.487. Patients of the therapeutic group had a higher ratio of successful liberation from mechanical ventilation (hazard ratio: 4.0 [95% CI 1.035–15.053]), p = 0.031 and more ventilator-free days (15 days [interquartile range IQR 6–16] versus 0 days [IQR 0–11]), p = 0.028 when compared to the prophylactic group.

Conclusion: Therapeutic enoxaparin improves gas exchange and decreases the need for mechanical ventilation in severe COVID–19.

Trial registration: REBEC RBR-949z6v.

1. Introduction

The primary clinical presentation of severe coronavirus disease 2019 (COVID-19) is an acute respiratory failure with extreme hypoxemia, which ultimately requires mechanical ventilation [1]. Recent clinical investigations found a high incidence of thrombotic complications in these patients, even with the standard anticoagulant thromboprophylaxis [2,3]. In addition to diffuse alveolar damage, several autopsy studies have demonstrated microthrombi in pulmonary circulation [4–8]. This microvascular thrombosis may contribute to impaired gas exchange in these patients.

Some observational studies have shown anticoagulation benefits with reduced mortality, mainly in patients requiring mechanical ventilation [9,10]. However, considerable levels of uncertainty remain about this therapy.

Our objective was to evaluate whether therapeutic anticoagulation could improve gas exchange compared to the standard anticoagulant thromboprophylaxis, reducing the need to maintain mechanical

Abbreviations: aPTT, activated partial thromboplastin time; ARDS, acute respiratory distress syndrome; BMI, body mass index; CI, confidence interval; CrCl, creatinine clearance; COVID-19, Coronavirus disease 2019; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DIC, disseminated intravascular coagulation; FiO2, fraction of inspired oxygen; ICU, intensive care unit; IQR, interquartile range; LMWH, low molecular weight heparin; PaO2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure; RT-PCR, reverse transcriptase-polymerase chain reaction; SAPS3, simplified acute physiology score 3; SIC, sepsis-induced coagulopathy score; SOFA, sequential organ failure assessment score; UFH, unfractionated heparin; ULN, upper limit of normal

* Corresponding author at: Department of Internal Medicine, Division of Emergency Medicine, Ribeirão Preto School of Medicine, São Paulo University, Rua Bernardino de Campos, 1000, Ribeirão Preto, São Paulo 14020-670, Brazil.
E-mail address: chmiranda@fmrp.usp.br (C.H. Miranda).

https://doi.org/10.1016/j.thromres.2020.09.026
Received 30 July 2020; Received in revised form 15 September 2020; Accepted 17 September 2020
Available online 21 September 2020
0049-3848/

ventilation in severe COVID-19 patients.

2. Methods

2.1. Study design

In this randomized, controlled, open-label, single-center, phase II study, we randomized patients with respiratory failure requiring mechanical ventilation and laboratorially confirmed SARS-CoV-2 infection to receive either therapeutic anticoagulation with enoxaparin or the standard anticoagulant thromboprophylaxis. The third arm of this study with therapeutic intravenous unfractionated heparin (UFH) was abandoned due to difficulties in adjusting the activated partial thromboplastin time (aPTT) during the pandemic. The study was approved by the Research Ethics Committee of our institution and followed the Declaration of Helsinki. The subjects or their relatives gave their written informed consent before enrollment in this study.

2.2. Patients

The inclusion criteria were patients with age over 18 years-old, SARS-CoV-2 infection confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR), presence of acute respiratory distress syndrome (ARDS) according to the Berlin definition [11], severe clinical presentation with respiratory failure requiring mechanical ventilation, D-dimer levels greater than 1000 μg/L; prothrombin time/international normalized ratio (INR) < 1.5; activated partial thromboplastin time (aPTT)/ratio < 1.5, and platelet count greater than 100,000/mm^3. The exclusion criteria were patients with age greater than 85 years-old, creatinine clearance (CrCl) < 10 mL/min, severe circulatory shock with a dose of norepinephrine higher than 1.0 μg/kg/min, chronic renal failure in renal replacement therapy, Child B and C chronic liver disease, advanced diseases, such as active cancer, heart failure with functional class III and IV (New York Heart Failure Association), chronic obstructive pulmonary disease using home oxygen, advanced dementia, significant disability from stroke or severe head injury, cardiorespiratory arrest, pregnant women, recent major surgery or severe trauma in the last 3 weeks, recent stroke in the last 3 months, active bleeding, blood dyscrasia such as hemophilia, Von Willebrand factor deficiency, participation in another clinical investigation, indication for therapeutic anticoagulation due to pulmonary embolism, and acute coronary syndrome.

For all patients, baseline characteristics and laboratory tests were retrieved from the medical records using a standardized data collection.

2.3. Randomization and procedure

We used blocked randomization, and the participants were randomized in a 1:1 ratio within two blocks of ten patients each. The patients were assigned to each treatment by drawing the sequential numbering of opaque envelopes containing the treatment allocation. The therapeutic enoxaparin group was allocated to receive subcutaneous (SC) enoxaparin with the dose according to age and adjusted daily by the creatinine clearance (CrCl) estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Patients under 75 years-old with CrCl > 50 mL/min received 1 mg/Kg BID; with CrCl between 30 and 50 mL/min: 0.75 mg/Kg BID; with CrCl between 10 and 30 mL/min: 1 mg/Kg OD; with CrCl < 10 mL/min: not included in the study. If their CrCl worsened at these levels (< 10 mL/min) during the investigation, they were transposed to intravenous unfractionated heparin (UFH) 24 h after the last dose of enoxaparin, which was adjusted according to the activated partial thromboplastin time (aPTT) aiming at a ratio between 1.5 and 2.0. Patients older than 75 years with CrCl > 50 mL/min received: 0.75 mg/Kg BID; with CrCl between 30 and 50 mL/min: 1 mg/Kg OD; with CrCl between 10 and 30 mL/min: 0.75 mg/Kg OD; with CrCl < 10 mL/min: similar to younger patients. Enoxaparin has a high degree of renal excretion and renal function can modify abruptly in critically ill patients. This drug accumulation, mainly in renal impairment and elderly patients, increases the bleeding risk. Because of this, we decided to use a more multitiered enoxaparin dose adjustment in these patients [12,13]. Therapeutic enoxaparin should be maintained for at least 96 h; however, we recommend maintaining it for up to 14 days. Anticoagulation could be suspended at any time if there was clinical evidence of bleeding. The maximum dose of enoxaparin allowed was 140 mg BID.

The standard thromboprophylaxis group was allocated to receive subcutaneous unfractionated heparin (UFH) at a dose of 5000 IU TID (if weight < 120 kg) and 7500 IU TID (if weight > 120 kg) or enoxaparin at a dose of 40 mg OD (if weight < 120 kg) and 40 mg BID (if weight > 120 Kg) according to the doctor's judgment.

2.4. Outcomes

The primary outcome was the variation in gas exchange over time evaluated through the ratio of partial pressure of arterial oxygen (PaO2) to the fraction of inspired oxygen (FiO2) at baseline, 7, and 14 days after randomization. This ratio was blindly calculated using the result of the arterial blood gas (ABG) analysis collected routinely around 6:00 am.

The secondary outcomes were the time until successful liberation from mechanical ventilation, the ventilator-free days (during the 28 days after inclusion in the study; numbers of days without mechanical ventilation), the variation in D-dimer levels collected at baseline during inclusion in the study and repeated 72–96 h later, all-cause 28-day mortality, in-hospital mortality, and the intensive care unit (ICU)-free days at 28 days.

To determine the successful liberation from mechanical ventilation, we considered the maintenance of spontaneous ventilation without the need for invasive mechanical ventilation support for a minimum of 72 h. All patients received the lung-protective ventilation strategy with low tidal volume. Each unit used its ventilator liberation protocol. The patients underwent prone positioning of at least 16 h if PaO2/FiO2 ratio < 150 mmHg. Other ventilator adjuncts to improve oxygenation in the setting of severe ARDS, such as inhaled nitric oxide and extracorporeal membrane oxygenation, were not available in our service.

The D-dimer measurement was performed through the automated assay VIDAS® D-dimer Exclusion II™ (bioMérieux SA, Lyon, France) with a cutoff of 500 μg/L.

The safety outcome analyzed was the occurrence of bleeding using the Thrombolysis in Myocardial Infarction (TIMI) bleeding criteria, which classified the bleeding in major, minor and requiring medical attention [14].

2.5. Statistical analysis

We used the Shapiro-Wilk test to evaluate the distribution of the continuous variables. We expressed the continuous variables with normal distribution as mean ± standard deviation or mean and its respective 95% confidence interval (95% CI), and other variables were expressed as the median and interquartile range (IQR). We used paired and unpaired *t*-test to assess the difference between two continuous variables with a normal distribution. The Mann-Whitney test was used to compare two continuous variables without normal distribution. We expressed categorical variables as frequency or percentage. We used Fisher's exact test to compare two categorical variables and the paired repeated measures one-way ANOVA test followed by Tukey's multiple comparison test to compare the PaO2/FiO2 ratio at baseline, 7, and 14 days. We constructed the Kaplan-Meier curves for survival analysis and compared them using the Cox regression-based test for equality of survival curves.

We defined the sample size in 10 patients per group, considering a paired sample, a significance level of 5%, a power of 85%, and an

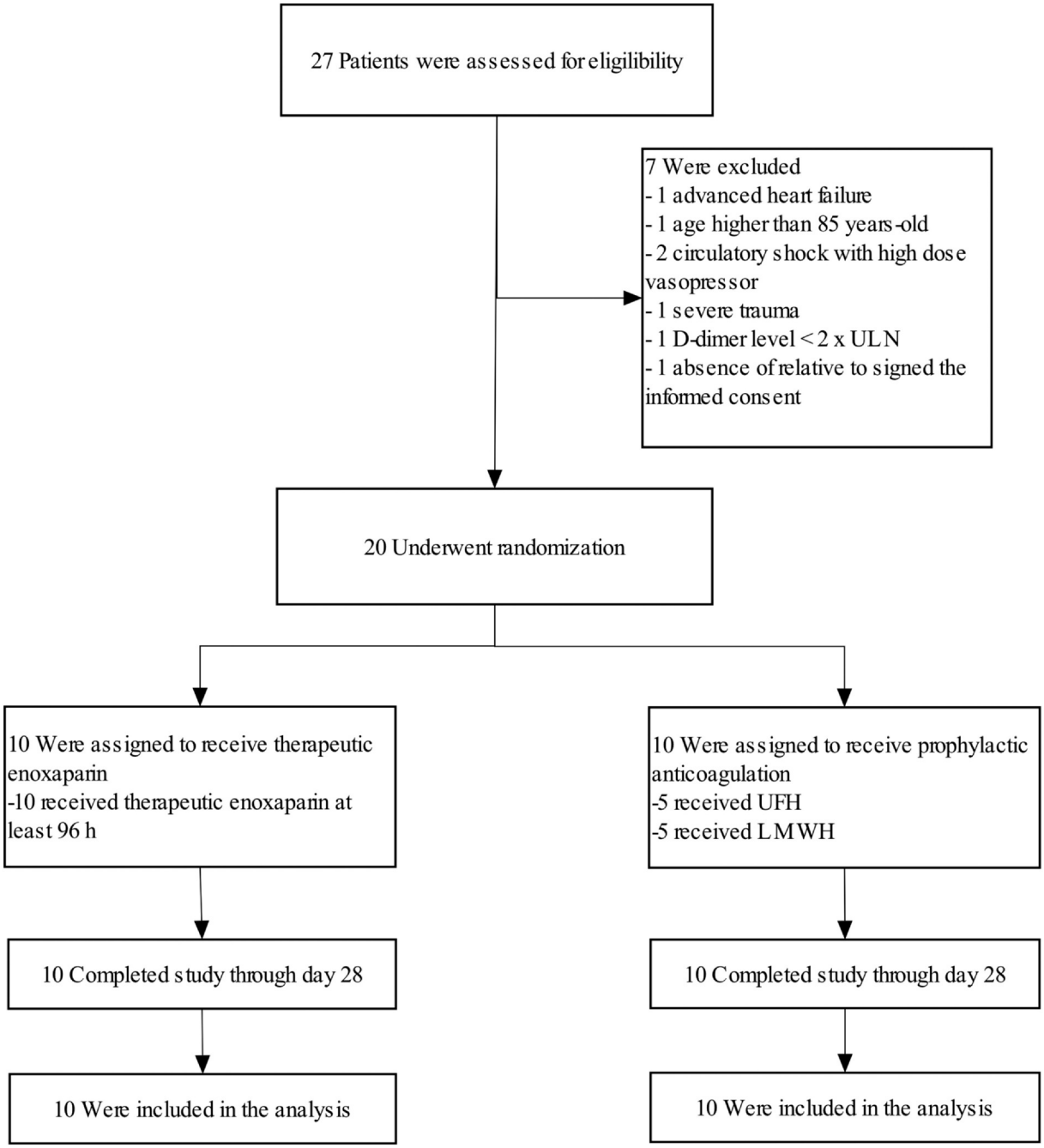

Fig. 1. Flow diagram showing the enrollment, randomization, and follow-up of the patients. ULN: upper limit of normal; LMWH: low molecular weight heparin; UFH: unfractionated heparin.

improvement in the PaO2/FiO2 ratio from 150 ± 50 (baseline) to 200 ± 50 (D14) with the therapeutic enoxaparin [15].

A two-tailed p-value less than or equal to 0.05 was considered statistically significant. Statistical analysis and graphs were performed using the GraphPad Prism software version 7.00 (California, USA) and the STATA software version 13 (College Station, TX, USA).

3. Results

Trial enrollment started in April 2020 and was completed in July 2020. Details about the disposition of the patients are provided in Fig. 1. The characteristics of the patients at baseline are described in Table 1. The baseline characteristics were similar between these two groups of patients. All patients were on mechanical ventilation and a similar proportion of patients underwent prone positioning in each group. They had a similar disease severity; the simplified acute physiology score 3 (SAPS3) and the sequential organ failure assessment score (SOFA) were not statistically different between the groups. Sixty percent of the patients in both groups received a low dose of norepinephrine to support the sedation and neuromuscular blocking agent. D-dimer levels were similar between the groups at baseline (3408 μg/L [95% CI 1283–5532] vs. 4176 μg/L [95% CI 1986–6365], p = 0.576). Despite the high D-dimer levels, the patients did not meet the criteria for disseminated intravascular coagulation (DIC).

We observed a statistically significant increase over time in the PaO2/FiO2 ratio among the patients in the therapeutic enoxaparin group (163 [95% CI 133–193] at baseline; 209 [95% CI 171–247] after 7 days; and 261 [95% CI 230–293] after 14 days), p = 0.0004. The

Table 1
Baseline characteristics.

	Prophylactic anticoagulation n = 10	Therapeutic enoxaparin n = 10	p value
Demographic			
Age (years), mean ± sd	58 ± 16	55 ± 10	0.529
Male gender, n (%)	7(70)	9(90)	0.264
Clinical features			
Fever, n (%)	8(80)	9(90)	0.531
Cough, n (%)	8(80)	9(90)	0.531
Dyspnea, n (%)	9(90)	9(90)	1.000
Myalgia, n (%)	4(40)	5(50)	0.653
Time from illness onset to hospital admission (days), mean ± sd	6 ± 3	7 ± 2	0.464
Medical history			
Diabetes mellitus, n (%)	3(30)	4(40)	0.639
Hypertension, n (%)	3(30)	4(40)	0.639
Cardiovascular disease, n (%)	1(10)	1(10)	1.000
Immunocompromise, n (%)	0(00)	1(10)	0.305
BMI (Kg/m^2), mean ± sd	34 ± 8	33 ± 8	0.828
Physical examination			
Systolic blood pressure (mmHg), mean ± sd	120 ± 25	125 ± 20	0.605
Diastolic blood pressure (mmHg), mean ± sd	72 ± 14	78 ± 12	0.319
Heart rate (beats per minute), mean ± sd	77 ± 14	80 ± 20	0.690
Mechanical ventilation, n (%)	10(100)	10(100)	1.000
Tidal volume (mL), mean ± sd	376 ± 67	419 ± 70	0.178
Tidal volume (mL per kg of PBW), mean ± sd	6.10 ± 0.57	6.10 ± 0.74	1.000
PEEP (cm of water), mean ± sd	12 ± 2	11 ± 3	0.408
Plateau pressure (cm of water), mean ± sd	24 ± 4	23 ± 4	0.293
Static compliance (mL/cm of water), mean ± sd	38 ± 13	37 ± 6	0.848
Respiratory rate (cycles per min), mean ± sd	25 ± 4	24 ± 3	0.323
FiO2 (%), mean ± sd	0.72 ± 0.23	0.78 ± 0.18	0.521
Prone positioning, n (%)	8(80)	7(70)	0.606
Previous condition before enrollment			
ICU stay before enrollment (days), median (IQR)	1(0–2)	0(0–2)	0.496
Prophylactic anticoagulation, n (%)	7(70)	4(40)	0.137
Therapeutic anticoagulation, n (%)	0(00)	0(00)	1.000
Drugs, n (%)			
Norepinephrine	6(60)	6(60)	1.000
Neuromuscular blocking agent	10(100)	10(100)	1.000
Corticosteroids	7(70)	7(70)	1.000
Hydroxychloroquine	1(10)	4(40)	0.121
Macrolide antibiotic	9(90)	9(90)	1.000
Antiplatelet agents	0(00)	0(00)	1.000
Remdesivir	0(00)	0(00)	1.000
Interleukin-6 inhibitors	0(00)	0(00)	1.000
Laboratory test			
Hemoglobin (g/dL), mean ± sd	13 ± 2	14 ± 2	0.163
White-cell count (per microliter), mean ± sd	11,107 ± 4178	7880 ± 2877	0.059
Platelet count (per microliter), mean ± sd	243,800 ± 48,622	203,700 ± 60,498	0.119
Creatinine clearance (mL/min), mean ± sd	59 ± 27	79 ± 19	0.074
Lowest creatinine clearance (mL/min)*, mean ± sd	46 ± 20	53 ± 20	0.412
Creatinine (mg/dL), mean ± sd	1.0 ± 0.4	1.0 ± 0.2	0.786
Peak creatinine (mg/dL)*, median (IQR)	1.43(1.30–1.59)	1.52(1.18–1.80)	0.705
D-dimer (μg/L), mean (95% CI)	3408(1283–5532)	4176(1986–6365)	0.576
Fibrinogen (mg/dL), median (IQR)	673(670–767)	774(685–946)	0.187
Prothrombin time (INR), median (IQR)	1.05(1.01–1.14)	1.05(0.99–1.17)	0.791
aPPT(Ratio), median (IQR)	1.14(1.05–1.17)	1.01(0.87–1.21)	0.364
C-reactive protein (mg/L), mean ± sd	16 ± 8	18 ± 7	0.504
Lactate (mg/dL), mean ± sd	1.8 ± 0.3	1.7 ± 0.4	0.556
PaO2/FiO2 ratio, mean ± sd	184 ± 53	163 ± 41	0.336
Scores, median (IQR)			
SOFA	10(8–11)	10(7–11)	0.877
SAPS 3	56(52–68)	56(51–68)	0.939
SIC	2(2–2)	2(2–3)	0.275
DIC	2(2–2)	2(2–2)	0.146

sd: standard deviation; CI: confidence interval; IQR: interquartile range; BMI: body mass index; kg: kilogram; PBW: predicted body weight; PEEP: positive end-expiratory pressure; PaO2: partial pressure of arterial oxygen; FiO2: fraction of inspired oxygen; ICU: intensive care unit; *during the 14 days after randomization; aPTT: activated partial thromboplastin time; INR: international normalized ratio; SOFA: sequential organ failure assessment score; SAPS 3: simplified acute physiology score 3; DIC: International Society of Thrombosis and Haemostasis criteria for disseminated intravascular coagulation; SIC: sepsis-induced coagulopathy score.

prophylactic anticoagulant group did not show statistically difference in the PaO2/FiO2 ratio over time (184 [95% CI 146–222] at baseline; 168 [95% CI 142–195] after 7 days; and 195 [95% CI 128–262] after 14 days), p = 0.487. Fig. 2A and B and Table 2. Only one patient of the prophylactic anticoagulation group died before the 14th-day. We considered the last PaO2/FiO2 ratio registered at the 12th-day after randomization as the surrogate of this patient's 14th-day ratio.

The patients in the therapeutic enoxaparin group showed a higher

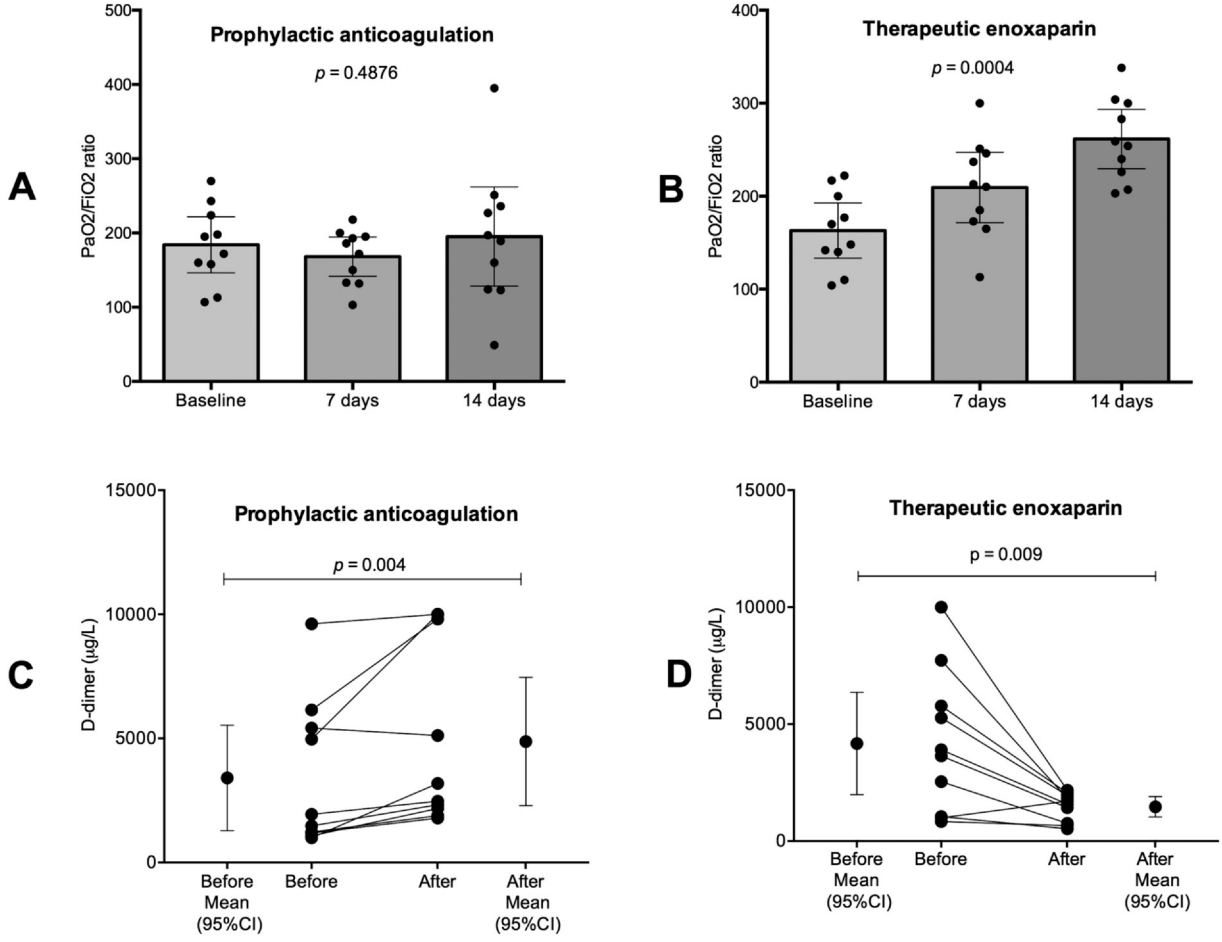

Fig. 2. Evolution of the gas exchange over time evaluated through the ratio of partial pressure of arterial oxygen (PaO2) to the fraction of inspired oxygen (FiO2) at baseline, 7, and 14 days after randomization in the patients of the prophylactic anticoagulation group (A) and the therapeutic enoxaparin group (B). Evaluation of D-dimer levels at baseline (before) and 72–96 h later (after) in the prophylactic anticoagulation group (C) and the therapeutic enoxaparin group (D). CI: confidence interval.

Table 2
Outcomes and adverse events.

	Prophylactic anticoagulation n = 10		Therapeutic enoxaparin n = 10		p–Value
Outcomes					
PaO2/FiO2 ratio, mean (95% CI)	p–Value*		p–Value*		
D0 (baseline)	184(146–222)	0.487	163(133–193)	0.0004	0.336
D7 (after 7 days)	168(142–195)		209(171–247)		0.060
D14 (after 14 days)	195(128–262)		261(230–293)		0.057
Ventilator-free days, median (IQR)	0(0 − 11)		15(6–16)		0.028
Number of prone positioning sessions, median (IQR)	2(2–3)		1(0–2)		0.048
All cause 28-day mortality, n (%)	3(30)		1(10)		0.264
In-hospital mortality, n (%)	5(50)		2(20)		0.160
ICU-free days, median (IQR)	0(0 − 10)		12(2 − 12)		0.067
Length of hospital stay; days, median (IQR)	30(23–38)		31(22–35)		0.838
Thrombotic events, n (%)	2(20)		2(20)		1.000
Adverse events, n (%)					
Major bleeding	0(00)		0(00)		
Minor bleeding	0(00)		2(20)		
Bleeding requiring medical attention	2(20)		4(40)		
Drop in hemoglobin levels (g/dL), mean (95% CI)	3(1–4)		4(3–6)		0.063
Drop in hemoglobin levels > 5.0 g/dL, n (%)	2(20)		4(40)		0.329

* Intragroup analysis; PaO2: partial pressure of arterial oxygen; FiO2: fraction of inspired oxygen; CI: confidence interval; IQR: interquartile range; ICU: intensive care unit.

ratio of successful liberation from mechanical ventilation (hazard ratio: 4.0 [95% CI 1.035–15.053]), p = 0.031 during the 28-day follow-up. Fig. 3 The ventilator-free days were higher in the therapeutic enoxaparin group (15 days [interquartile range IQR 6–16]) compared to the prophylactic anticoagulation group (0 days [IQR 0–11]), p = 0.028.

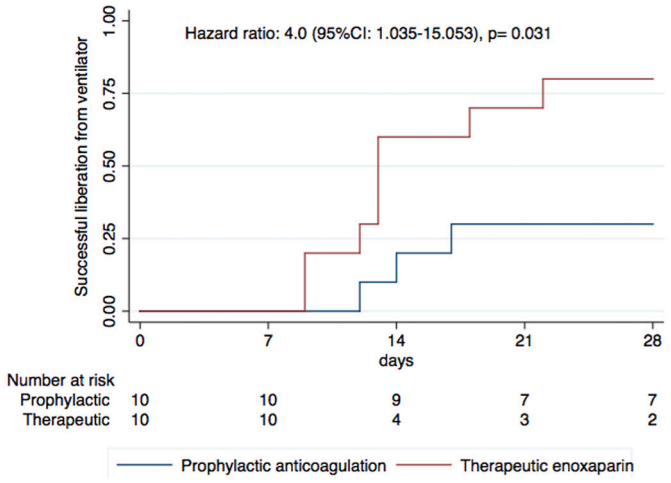

Fig. 3. Kaplan-Meier curve comparing the cumulative incidence of successful liberation from mechanical ventilation in the therapeutic enoxaparin and prophylactic anticoagulation groups at 28 days of follow-up. CI: confidence interval.

The D-dimer levels evidenced a statistically significant decrease over time in the therapeutic enoxaparin group (4176 μg/L [95% CI 1986–6365] vs. 1469 μg/L [95% CI 1034–1904]), p = 0.009, and statistically significant increase over time in the prophylactic anticoagulation group (3408 μg/L [95% CI 1283–5532] vs. 4878 μg/L [95% CI 2291–7465]), p = 0.004. Fig. 2C and D. The time difference between these two measurements was not statistically different between the groups (3.9 ± 1.2 days in the therapeutic enoxaparin group vs. 4.3 ± 1.2 days in the prophylactic anticoagulation group), p = 0.457.

We did not observe differences in the all-cause 28-day mortality rate between the therapeutic enoxaparin group 1/10 (10%) and prophylactic anticoagulation group 3/10 (30%), p = 0.264, and in-hospital mortality rate 2/10 (20%) versus 5/10 (50%), p = 0.160, respectively. We also did not observe any statistically significant difference in the ICU-free days (12 days [IQR 2–12] in the therapeutic enoxaparin group vs. 0 days [IQR 0–10] in the prophylactic anticoagulation group), p = 0.067. Table 2.

The median of the therapeutic enoxaparin treatment duration was 14 days, ranging from 9 to 14 days. Transposition to intravenous unfractionated heparin (UFH) was unnecessary in any patient in the therapeutic enoxaparin group due to worsening renal function during the 14 days after randomization.

We did not actively investigate the occurrence of thrombotic events in these patients. Despite this, there was a documentation of two thrombotic events in each group (one deep vein thrombosis (DVT) and one pulmonary embolism in the prophylactic group and two DVT in the therapeutic group).

As for the safety outcomes, we did not register any major bleeding in either group. Two patients (20%) in the therapeutic enoxaparin group experienced minor bleeding (one hematuria and one femoral arterial catheter bleeding). We observed bleeding requiring medical attention in four patients (40%) of the therapeutic enoxaparin group and two patients (20%) of the prophylactic anticoagulation group; these cases were associated to a significant drop in hemoglobin levels (> 5 g/dL) without any clinically overt sign of hemorrhage. Hemoglobin levels dropped in both groups (4 g/dL [95% CI 3–6] in the therapeutic enoxaparin group and 3 g/dL [95% CI 1–4] in the prophylactic anticoagulation group), p = 0.063. The deaths of the two patients in the therapeutic enoxaparin group were secondary to healthcare-associated infection. Table 2.

4. Discussion

In this randomized clinical trial, therapeutic enoxaparin resulted in improved gas exchange over time, decreased D-dimer levels, and a higher ratio of successful liberation from mechanical ventilation after respiratory failure in severe COVID-19 patients.

Several studies have performed autopsies on patients who died from severe COVID-19 and found microthrombi in pulmonary circulation in most of them [4–8]. In one of these investigations, small thrombi in pulmonary circulation were nine times more prevalent in patients with COVID-19 compared to patients who died of influenza (p < 0.001) [5]. Additionally, new vessel growth was 2.7 times higher in COVID-19 than in influenza (p < 0.001) [5]. In addition to the diffuse alveolar damage, which is the hallmark of this disease, microvascular thrombosis in pulmonary circulation could compromise gas exchange, contributing to the significant hypoxemia observed in these patients. The principal hypothesis investigated in this study was whether therapeutic enoxaparin could improve these microvessels' patency, leading to improved blood oxygenation.

Our investigation showed significant improvement in the PaO2/FiO2 ratio overtime in the therapeutic enoxaparin group. On the other hand, we did not observe a significant improvement in the prophylactic anticoagulation group. Moreover, the frequency of prone positioning was similar in both groups, since this intervention showed to be effective in ameliorating blood oxygenation in acute respiratory failure due to COVID–19 [16]. Lu et al. [17] demonstrated that the oxygen saturation to the fraction of inspired oxygen (SpO2/FiO2) ratio was an important prognostic marker for critically ill COVID-19 patients, with a unit decrease in the marker corresponding to 1.82–fold increase in mortality risk [95% CI 1.56–2.13]. The dynamic profile of the SpO2/FiO2 ratio presented a rising trend in survivors and a sharp decrease over the first few days in non-survivors.

Two observational studies have demonstrated the clinical benefit of anticoagulant in COVID-19 patients. A retrospective study of Tang et al. [9] achieved a low 28-day mortality in severe Covid-19 patients with the use of anticoagulant therapy for 7 days or more, especially the ones with high sepsis-induced coagulopathy score (≥4) or high D-dimer (≥3.0 mg/L). In another retrospective study, Paranjpe et al. [10] verified in 395 patients with COVID-19, who required mechanical ventilation, a lower in-hospital mortality for those treated with systemic anticoagulation (29.1%) than those who did not receive this treatment (62.7%). In a multivariate proportional hazard model, a longer duration of systemic anticoagulation was associated with reduced risk of mortality (adjusted hazard ratio: 0.86 per day; [95% CI 0.82–0.89]), p < 0.001.

Despite evidence about coagulation activation and impaired fibrinolysis leading to fibrin deposition in the alveolar compartment in non-COVID-19 ARDS patients [18], data about therapeutic anticoagulation's efficacy and safety is very limited in these patients [19]. Some recent investigations showed that low molecular weight heparin (LMWH) could reduce acute lung injury (ALI) in experimental models [20,21]. A meta-analysis including nine randomized clinical trials involving 465 patients displayed that adjunctive LMWH treatment reduced the 28-day mortality (relative risk 0.63 [95% CI 0.41–0.96]) as well as increased the PaO2/FiO2 ratio (weighted mean difference 74.48 [95% CI 52.18–96.78] in ALI/ARDS patients and 44.06 [95% CI 17.05–71.08] in only ARDS patients) [15]. However, these individual trials' methodological quality was relatively low, and they were available only in Chinese databases.

D-dimer levels also appear to be a predictor of death in COVID-19 patients. Zhou et al. [22] (n = 191) reported a significantly higher D-dimer of around 9-fold in non-survivors than survivors with severe COVID-19 (81% vs. 24%, p < 0.001). Tang et al. [23] had a similar finding, with a higher D-dimer levels in non-survivors (n = 21) compared to survivors (n = 162), (2.12 μg/mL [IQR 0.77–5.27] vs. 0.61 μg/mL[IQR 0.35–1.29], respectively), p < 0.001. Our

investigation demonstrated a statistically significant decrease in D-dimer levels in the therapeutic enoxaparin group. On the other hand, we observed a statistically significant increase in D-dimer levels in the prophylactic anticoagulant group. Yu et al. [24] showed that D-dimer levels decreased in patients with good clinical prognosis.

COVID-19 is associated with a high prevalence of thrombotic events in both territories (macro and microvascular) [2,5]. The mechanism responsible for this coagulopathy in COVID-19 has not been fully elucidated; however, it seems that the dysregulation of the immune response triggered by pro-inflammatory cytokines, lymphocyte cell-death, hypoxia, and endothelial damage are involved [25]. Recently, Khider et al. [26] showed that curative anticoagulation could prevent COVID-19-associated coagulopathy and endothelial lesion.

In our investigation, the therapeutic enoxaparin was prescribed for a median of 14 days. We considered this extended time because it is necessary to solve the inflammatory process before suspending the anticoagulant therapy.

Despite the COVID-19-associated coagulopathy, bleeding tendency is uncommon in this disease [25]. In comparison with bacterial-sepsis induced coagulopathy, the prolongation of prothrombin time and activated partial thromboplastin time is less frequent, and thrombocytopenia is relatively uncommon in COVID-19 [25]. The incidence of clinically evident bleeding was low in both groups, including the group with therapeutic enoxaparin. Paranjpe et al. [10] reported 3.0% of bleeding events among those receiving systemic anticoagulation compared to 1.9% among those who did not receive this treatment ($p = 0.2$). In the investigation of Al-Samkari et al. [27], the overall and major bleeding risks were 4.8% and 2.3%, respectively, with the most varied anticoagulation protocols in COVID-19 patients. We observed a drop in hemoglobin levels in both groups without any clinical evidence of bleeding in most patients. The inflammatory status might explain this anemia because the cytokines reduce erythropoietin levels, affect iron homeostasis by multiple mechanisms, directly inhibit erythroid cell differentiation and proliferation, and reduces red blood cell survival [28]. As a result, we considered that an isolated drop in hemoglobin levels without any clinical evidence of bleeding is not an indication to suspend therapeutic enoxaparin in these severe patients.

The main limitation of our investigation is that this single center study with a small sample size did not have sufficient power to assess a difference in mortality between the two groups. The PaO2/FiO2 ratio assessment has limitations in quantifying pulmonary dysfunction improvement; however, the increase in the PaO2/FiO2 ratio in the therapeutic enoxaparin group in our investigation was accompanied by a higher ratio of liberation from mechanical ventilation and higher ventilator-free days. The incidence of bleeding may be higher in patients older than those included in this study. Moreover, critically ill COVID-19 patients could have the documentation of clinically overt bleeding impaired due to difficulties in performing imaging tests. Despite this, five out of six patients with hemoglobin drop higher than 5 g/dL recovered from the disease and received hospital discharge without any evidence of significant hemorrhage such as intracranial bleeding.

5. Conclusion

This open-label, controlled, randomized clinical trial demonstrated that therapeutic enoxaparin improved gas exchange over time and increased the ratio of successful liberation from mechanical ventilation. After these results, a larger clinical trial is urgently needed to evaluate the anticoagulant therapy in severe COVID-19 patients.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interets

All authors declare no conflicts of interest.

Availability of data and material

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

Author's contributions

ACBL–acquisition of data, analysis and interpretation of data, draft the article, final approval of the last version. DAES–acquisition of data, analysis and interpretation of data, draft the article, final approval of the last version. MCS–acquisition of data, critical revision of the manuscript, final approval of the last version. RNG–acquisition of data, critical revision of the manuscript, final approval of the last version. LBA–acquisition of data, critical revision of the manuscript, final approval of the last version APF–critical revision of the manuscript, final approval of the last version. CHM–conception and design of the study, acquisition of data, analysis and interpretation of data, draft the article, final approval of the last version.

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