HEMOSTASIS AND THROMBOSIS

Original article

Clinical evaluation of the safety and efficacy of enoxaparin in patients with COVID-19

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Background - Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), increases thrombotic risk in hospitalised patients. The rate of thrombosis in patients with COVID-19 is unclear. The role of heparin, frequently used in the management of hospitalised patients, also needs to be clarified. In this study, we investigated the efficacy and safety of enoxaparin given at prophylactic or therapeutic dose in hospitalised patients with COVID-19, and evaluated its role in the development of disease in terms of mortality, and incidence of thrombotic and bleeding events.

Material and methods - We included 141 patients with SARS-CoV-2 infection, admitted to five different wards (one intensive care unit, 2 sub-intensive care units, and 2 general infectious disease units) of Cotugno Hospital, a tertiary care hospital in Naples, Italy, between March and May 2020.

Results - Over a median time of 17 days (IQR 11-25), enoxaparin was given to 90/141 patients (63.8%) of whom 65 took a prophylactic and 25 a therapeutic dose. We documented 14 episodes of thrombosis (9.9%); almost all were cases of pulmonary embolism. No significant difference in terms of thromboembolic prevention was found between those patients not receiving anticoagulants and those on prophylactic or therapeutic dose of enoxaparin. Five episodes of major bleeding occurred (3.5%); therapeutic dose of enoxaparin was associated with a greater bleeding risk than prophylactic dose (p=0.002). During follow-up, 31 patients (22%) died; these were mostly elderly men with two or more comorbidities at admission. No advantages of enoxaparin, either as prophylaxis or at high doses, in terms of mortality were observed. At multivariate analysis, low estimated glomerular filtration rate, and high total bilirubin and fasting hyperglycemia were independently associated with a higher mortality.

Discussion - We did not observe advantages in terms of either thromboembolic prevention or mortality of enoxaparin, which however was more frequently used in patients with more severe disease. Prophylactic enoxaparin was not seen to be associated with bleeding risk.

Keywords: *enoxaparin, COVID-19, thrombosis.*

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INTRODUCTION

Coronavirus disease-2019 (COVID-19) is a viral illness caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and a pandemic has been declared by the

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World Health Organization (WHO)[1]. It is now well recognised that patients with COVID-19 are at risk of venous and arterial thromboses because SARS-CoV-2 triggers an intense inflammatory response. The primary SARS-CoV-2 infection initiates alveolar injury with activation of inflammatory response and production of inflammatory cytokines, including interleukin-6 (IL-6). It also stimulates activation and recruitment of mononuclear cells and neutrophils. The release of large quantities of inflammatory mediators that stimulate the expression of tissue factor on monocytes/macrophages and vascular endothelial cells, as well of hormones and immunoglobulins in severe or critically ill patients, may lead to an increase in blood viscosity, stasis and activation of coagulation cascade. Indeed, SARS-CoV-2, through angiotensin 2 receptor (ACE2), infects endothelial cells leading to increased levels of angiotensin II and tissue factor expression[2]. The increase in angiotensin II levels reduces the production of nitric oxide, resulting in vasoconstriction and venous stasis. Tissue factor expression stimulates the coagulation cascade. In classical COVID-19-associated coagulopathy (CAC), patients often initially present with increased fibrinogen levels and increased D-dimers, but with little alteration in prothrombin time or platelet count. All these pathophysiological changes may lead to the development of disseminated intravascular coagulopathy (DIC) and microvascular thrombosis. The rate of venous thromboembolism (VTE) in patients hospitalised for COVID-19 is not known; incidence rates reported in the literature vary between 11.7 and 69%[3-8]. This wide variability could be due to the selection of patients and the study setting, the type of events analysed, whether systematic screening for VTE was carried out or not, and the use of any thromboprophylaxis[9]. Concomitant factors that can increase the risk of VTE in hospitalised patients are prolonged immobilisation, comorbidity (such as hypertension, diabetes, obesity, cardiovascular disease), age, previous history of VTE, and classical genetic thrombophilia[10]. The anticoagulants most frequently used in hospital settings are heparin-based. Low molecular weight heparins (LMWH) or unfractionated heparin (UFH) should be preferred over direct oral anticoagulants (DOACs) due to possible drug-drug interactions with concomitant antiviral and antibacterial treatment[11].

The efficacy of anticoagulant therapy and the optimal dosage to prevent thromboembolic events in COVID-19 patients have not yet been defined. Patell *et al*. conducted a systematic review and analysed studies reporting thrombotic (arterial or venous) events according to anticoagulation status. A statistically significant reduction in thromboembolic events was detected in patients receiving standard-dose pharmacological thromboprophylaxis (LMWH, UFH, DOACs) compared with patients who did not receive therapy. However, there was no statistically significant reduction in the incidence of thromboembolic events between those patients receiving prophylactic (standard or intermediate-dose) anticoagulant therapy and those receiving therapeutic-dose anticoagulation[12]. Pesavento *et al*. showed no difference in the rate of thromboembolic events between those patients who received sub-therapeutic doses of heparins or fondaparinux or conventional prophylactic doses[13]. Martinelli *et al*. observed lower rates of VTE in patients hospitalised in intensive care units (ICU) receiving high-dose regimens of enoxaparin compared to patients receiving standard-dose prophylaxis[14]. Therefore, because of limited data from specific controlled/randomised studies, the optimal dosage of LMWH in this setting is still unknown and remains a subject of debate.

In this retrospective study, we reviewed clinical data from the first wave of pandemic, investigating the efficacy and safety of enoxaparin given at prophylactic or therapeutic dose in hospitalised patients with COVID-19. We evaluated its possible role in the development of disease in terms of mortality, and the incidence of thrombotic (pulmonary embolism, deep venous and arterial thrombosis) and bleeding events.

MATERIALS AND METHODS

Study cohort

This was a retrospective, observational study of 141 consecutive patients with laboratory-confirmed SARS-CoV-2 infection, admitted to five different wards (one ICU, 2 sub-ICUs, and 2 general infectious disease units) of Cotugno Hospital, a tertiary care hospital in Naples, Italy, between March and May 2020.

Confirmation of SARS-CoV-2 infection was defined as a positive result of real-time reverse

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transcriptase-polymerase chain reaction (RT-PCR) assay of nasal and pharyngeal swabs, according to WHO laboratory guidelines. For the purpose of the study, we only considered patients receiving enoxaparin at any time during their hospital stay. Patients under 18 years of age and those who had received different thromboprophylaxis agents (fondaparinux or UFH) during hospitalisation were not included.

All study procedures were carried out in accordance with international guidelines, and the standards on human experimentation of the Ethics Committee of the 1975 Helsinki Declaration (as revised in 2013) and subsequent revisions. The study protocol was approved by the Ethics Committee of AORN dei Colli (deliberation AOC/0016233-2020 of 03/06/2020). In cases in which it was not possible to ask patients for consent to the processing of data for ethical and administrative reasons, it is to be noted that the study fell within the scope and purposes specified by the Guarantor for the Protection of Personal Data (General Authorisation for the processing of personal data carried out for scientific research purposes and General Authorization for the processing of genetic data [15 December 2016] Official Journal n. 303 of 29 December 2016).

Data collection

Study data and clinical information were collected and managed by medical staff using electronic clinical records. For each patient, the following baseline data were collected: age, sex, main comorbidities, admission ward (general ward, sub-ICU or ICU), body temperature, lung function indices and type of respiratory support therapy (low-flow oxygen, high-flow oxygen, continuous positive airway pressure, or mechanical ventilation), radiology examinations (X-ray or computed tomography [CT]), clinical or radiological evidence of thrombosis or bleeding and type of event, laboratory tests, including complete blood count, coagulation tests, liver and kidney function tests, and inflammatory markers. Moreover, drugs used as supportive therapy of COVID-19 were recorded (antivirals, antibiotics or anti-inflammatories), together with anti-platelet and antithrombotic drugs. The above-mentioned assessments were also registered during the entire hospital stay according to the available data.

Timing of initiation and dosing regimen of enoxaparin (prophylactic/standard or intermediate/or therapeutic doses), chosen by the attending physicians according to the patient's clinical status and history, perceived thromboembolic risk and evidence of arterial or venous thrombosis, were registered. Enoxaparin doses of 40 mg or 60 mg once a day (OD) were defined as prophylactic and doses of 40 mg, 60 mg and 80 mg twice a day (BID) as therapeutic.

Study outcomes

The occurrence of pulmonary embolism (PE) was diagnosed at CT angiography, while upper and lower extremity deep vein and arterial thrombosis were considered according to the clinical or ultrasound assessment reported in medical records; ultrasonography was not available in all patients. Major bleeding and clinically relevant non-major bleeding were defined according to the definition of the International Society of Thrombosis and Haemostasis (ISTH)[15].

Further factors affecting the mortality of the patients studied were taken into account and the incidence of other clinical complications was considered, such as fasting hyperglycemia (above 126 mg/dL, as at diabetic level), acute liver injury (ALI), acute kidney injury (AKI), and disseminated intravascular coagulation (DIC). ALI was defined as an increase in alanine aminotransferase (ALT) more than twice the upper limit of normal (ULN) or an increase in conjugated bilirubin or a combined increase in aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin, provided that one of them was over twice the ULN[16]. For AKI, we used diagnostic criteria established by the major international guidelines[17]; diagnostic criteria established by the Scientific and Standardization Committee of the ISTH were adopted for DIC[18].

Statistical analysis

Continuous variables are presented as median with range; categorical/nominal data are presented as number and percentage. Fisher's exact test and Pearson's χ^2 test were used to assess statistical significance of the differences between nominal groups of variables, followed by Bonferroni method for further analysis. Logistic regression analysis of independent predictors of mortality was performed by block, entering all variables significantly associated with each of these results into the univariate analysis. Overall survival according to enoxaparin use was estimated using the Kaplan-Meier method. Duration of follow-up was calculated from the date of admission to the date of death or the last available follow-up. The level of significance was fixed at 5% and all tests were two-tailed.

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All analysis was performed using the statistical software for Windows SPSS 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of the study population at baseline

Overall, 141 patients were enrolled in the study. Their clinical characteristics at baseline are summarised in **Table I**. Median age was 59.7 years (interquartile ratio [IQR], 24-88) and 104 were men (73.8%). Forty-four (31.2%) had no pre-existing comorbidities, 46% suffered from one chronic underlying disease, while 51% had more than one comorbidity, mainly hypertension (41.1%), diabetes (15.6%), and cardiac diseases (14.2%). At baseline, 11 (7.8%) patients

Table I - *Baseline characteristics of enrolled patients (N=141)*

Baseline features of study patients	
Number	141
Age, years	59.7 [24-88]
Male gender	104 (73.8)
Length of stay, days	16.7 [1-49]
Body temperature, °C	37.7 [35-40]
Cough	88 (62.4)
COVID-19 pneumonia severity at admission	
Need of low flow oxygen	61 (43.3)
Need of high flow oxygen	0 (0.0)
Continuous Positive Airway Pressure (CPAP)	11 (7.8)
Mechanical ventilation	6 (4.2)
Comorbidities	
No comorbidity	44 (31.2)
One comorbidity	46 (32.6)
Multi comorbidity	51 (36.2)
Comorbidity type	
Cardiac	20 (14.2)
Diabetes mellitus	22 (15.6)
Renal	11 (7.8)
Arterial hypertension	58 (41.1)
Neurological	10 (7.1)
Respiratory	18 (12.8)
Hepatic	11 (7.8)
Neoplasia	5 (3.5)
Obesity	12 (8.5)
Smoker	14 (9.9)
Other	4 (2.8)
COVID-19 specific treatments	
Antibiotics	135 (95.7)
Antivirals:	
Lopinavir/Ritonavir	80 (56.7)
Darunavir/Cobicistat	6 (4.2)
Anti-inflammatories:	
Hydroxychloroquine	99 (70.2)
Tocilizumab	13 (9.2)
Sarilumab	4 (2.8)
Steroids	5 (3.5)
Dose of enoxaparin administered	
40 mg OD	28 (48.3)
60 mg OD	13 (22.4)
40 mg BID	7 (12.1)
60 mg BID	8 (13.8)
80 mg BID	2 (3.4)
Antiplatelet therapy	
None	131 (92.9)
SAPT[a]	9 (6.4)
DAPT[b]	1 (0.7)
Intensive care unit	11 (7.8)
Sub-intensive care unit	11 (7.8)
Chest X-Ray (RX)	13 (9.2)
Chest computed tomography (TC)	126 (89.4)
White blood cells, cells/µL	6,730 [1,920-51,000]
Neutrophil, cells/µL	5035 [1,070-19,650]
Lymphocytes, cells/µL	943 [200-3,170]
Eritrocytes, cells10^3/µL	4710 [4.27-6,010]
Platelets, cells10^3/µL	189 [0.33-1,250]
Haemoglobin, g/dL	14 [8.4-18]
Haematocrit, %	40.4 [26.1-51]
Creatinine, mg/dL	0.9 [0.5-12.0]
Glucose, mg/dL	105 [16-343]
Troponin I, ng/mL	4.5 [0.01-2200]
International Normalised Ratio (INR)	1.17 [0.96-4.07]
Prothrombin time (PT), %	78.5 [0.1-106]
Partial thromboplastin time (PTT), seconds	35.8 [21.8-3405]
D-Dimer, ng/mL	476 [52-36860]
Fibrinogen, mg/dL	560.7 [247-955]
C-reactive protein, mg/dL	9.1 [0.4-37.0]
Ferritin, ng/mL	660.3 [20.6-4,440]
Blood urea nitrogen (BUN), mg/dL	34.3 [9-223]
Aspartate aminotransferase (GOT), U/L	36 [10-213]
Alanine aminotransferase (GPT), U/L	31 [7-158]
Gamma glutamyl transferase (GGT), U/L	39.3 [8-259]
Albumin, g/dL	4.01 [2.6-5.3]
Direct bilirubin, mg/dL	0.27 [0.1-1.0]
Total bilirubin, mg/dL	0.64 [0.2-15.0]
Lactate dehydrogenase (LDH), U/L	317 [136-1,222]
Creatine kinase (CK), U/L	115.2 [9.0-2,750]

Data are median [range] or number (percent).
OD: once daily; BID: twice daily; SAPT: single antiplatelet therapy; DAPT: dual antiplatelet therapy.

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needed Continuous Positive Airway Pressure (CPAP), while only 6 (4.2%) received mechanical ventilation. Eleven patients (7.8%) required hospitalisation in an ICU, and a further 11 patients were admitted to a sub-ICU. In almost all patients (139/141, 98.6%), radiologic imaging to diagnose and stage COVID-19 pneumonia was performed. Sixty-one percent of patients received antivirals, 95.7% antibiotic therapy, and 3.5% steroids. Aspirin as single antiplatelet drug was administered to 6.4% of the patients, while only one patient received aspirin in combination with ticagrelor. At baseline, 58 patients (41.1%) received enoxaparin, of which 41 and 17 at prophylactic and therapeutic dose, respectively.

Low molecular weight heparin use during hospitalisation

Study patients had a median length of hospital-stay of 17 days (IQR 11-25). During this period, enoxaparin was given to 90/141 patients (63.8%), of whom 65 (72.2%) took a prophylactic dose and 25 (27.8%) a therapeutic dose. In the prophylactic group, a dose of 40 mg OD and 60 mg OD was given to 44 and 21 patients, respectively. Of the 25 patients treated with a therapeutic dose of enoxaparin, 36% received 40 mg BID, 48% 60 mg BID, and 16% 80 mg BID.

Thrombotic events

In our study cohort, we documented 14 episodes of thrombosis (9.9%), including one arterial thrombosis of the lower extremities and 13 cases of PE, of which 12 involved segmental/subsegmental arteries and only one involved main/lobar arteries. Of the 14 affected patients, 8 (57.1%) required intensive or sub-intensive care and 6 were managed on a general care ward. Two episodes of thrombosis were diagnosed at baseline, 5 within the first week after admission, 2 after 14 days following hospitalisation, 2 after 3 weeks, and 3 after 4 weeks. Four of the 14 patients with thrombosis died (28.6%).

At the time of the documented event, 4 patients were not receiving any anticoagulant therapy, 4 were on prophylactic dose of enoxaparin (40 mg OD in 2 cases, and 60 mg OD in the others), and 6 were already on therapeutic doses (40 mg BID in 2 cases, 60 mg BID in other 2 cases, and 80 mg BID in the others), in 5 of them just before clinical suspicion of thrombosis based on increased D-dimer levels with a median value of 5,593 (IQR 1,253-11,569). No significant statistical difference in terms of thromboembolic prevention between patients not receiving anticoagulant therapy and those on both prophylactic or therapeutic dosage of enoxaparin (**Table II**) was observed. Instead, there was a lower incidence of thrombosis in patients on prophylactic doses than in those on higher doses of enoxaparin (p=0.012) (**Table II**).

Bleeding events

During observation, 5 episodes of major bleeding occurred (3.5%), including haematomas in the upper limbs, ilio-psoas haematoma, anterior abdominal wall haematoma, macroscopic haematuria and bronchial haemorrhage. Three patients needed intensive/sub-intensive care while 2 remained on a general care ward. Four out of 5 patients were over 70 years of age, 2/5 had decompensated cirrhosis (both had criteria for DIC), while none had a history of previous bleeding. One patient had a chronic kidney disease (CKD) with an estimated glomerular filtration rate (eGFR) <30 mL/min, whereas another 2 had experienced AKI before bleeding. At the time of the haemorrhagic event, 4 patients were on optimal or sub-optimal therapeutic dose of anticoagulants because of atrial fibrillation (n=1), an increase in D-dimer value >10 ULN in association with a worsening of respiratory function (n=1), previous diagnosis of DIC (n=1), or PE (n=1). Specifically, one patient was not receiving anticoagulant, 2 patients were on enoxaparin 40 mg BID, and another 2 were receiving 60 mg BID. Furthermore, one patient was

Table II - *Dose of enoxaparin administered in patients who experienced thromboembolic and bleeding events*

	Enoxaparin			
	No therapy	**Prophylactic**	**Therapeutic**	**^p-value**
Thromboembolic events, n=14 (%)	4 (28.6)	4 (28.6)	6 (42.9)	0.012*
Bleeding events, n=5 (%)	1 (20.0)	0 (0.0)	4 (80.0)	0.002*

^p-value was generated by Pearson χ^2 test . *Statistical significant difference between patients receiving prophylactic *vs* therapeutic enoxaparin.

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taking aspirin. Four of 5 patients with bleeding events (80%) died.

Although 4/5 events occurred in patients receiving a high dose of enoxaparin, there was no significant difference in the incidence rate of bleeding between those patients receiving and those not receiving enoxaparin (**Table II**). However, we found that a therapeutic dose of enoxaparin was associated with a greater haemorrhagic risk than a prophylactic dose (p=0.002).

Mortality

During a median follow-up of 17 days (IQR 11-25), 31 patients (22%) died with a median time of survival of 16 days (IQR 9-24). They were mostly men (87.1%); median age 73 years. We observed lower mortality rates (p<0.001) in patients with no or one comorbidity than in those with 2 or more (*data not shown*); at multivariate regression analysis, kidney, respiratory and liver diseases were independently associated with mortality (**Table III**).

Ten of 31 deceased patients (32.2%) had been admitted to a general care ward, 5 (16.1%) had been admitted to a sub-ICU, and 16 (51.6%) to an ICU. Among the 31 patients who died, 4 episodes of major bleeding (one upper extremities haematoma, one anterior abdominal wall haematoma, one macroscopic and one bronchial haemorrage)

Table III - *Comorbidities associated with mortality with multivariate logistic regression analysis*

Type of comorbidity	Exitus		Univariate analysis			Logistic regression		
	Alive	Dead	Odds Ratio	(95% CI)	^p-value	Odds Ratio	(95% CI)	^p-value
Cardiac disease								
No	98	23	2.84	(1.04-7.74)	0.045	2.18	(0.63-7.47)	0.213
Yes	12	8						
Chronic kidney disease								
No	105	25	5.04	(1.42-17.8)	0.015	4.44	(1.10-17.9)	0.036
Yes	5	6						
Hypertension								
No	70	13	2.42	(1.07-5.45)	0.039	1.72	(0.67-4.43)	0.256
Yes	40	18						
Diabetes								
No	97	22	3.05	(1.16-8.03)	0.027	1.92	(0.60-6.11)	0.264
Yes	13	9						
Neurological disorder								
No	103	28	1.57	(0.38-6.49)	0.458			
Yes	7	3						
Chronic respiratory disease								
No	102	21	6.07	(2.14-17.2)	0.001	7.16	(2.30-22.2)	0.001
Yes	8	10						
Malignancy								
No	107	29	2.46	(0.39-15.4)	0.303			
Yes	3	2						
Obesity								
No	103	26	2.83	(0.83-9.63)	0.136			
Yes	7	5						
Smoking abuse								
No	99	28	1.03	(0.27-3.97)	1.000			
Yes	11	3						
Chronic liver disease								
No	105	25	5.04	(1.42-17.8)	0.015	4.50	(1.07-18.9)	0.040
Yes	5	6						
Others								
No	107	30	1.18	(0.11-11.8)	1.000			
Yes	3	1						

eGFR: estimated Glomerular Filtration Rate; ALT: alanine aminotransferase; ULN: upper limit of normal. ^p-value was generated by Fisher's exact test.

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and 4 thrombotic events (3 cases of pulmonary microembolism and one case of arterial thrombosis of the lower extremities) occurred. Specifically, when major bleeding events happened, all 4 patients were receiving a therapeutic dose of enoxaparin; at time of diagnosis of thrombotic events, 3 patients were not receiving any dose of anticoagulant, while one had already been started on a therapeutic dose of enoxaparin due to a remarkable increase in D-dimer (>8,000 ng/mL). None suffered from minor haemorrhages. Thirty-six percent (11/31) of patients presented marked hyperglycemia (glucose serum level ≥200 mg/dL), 29% (9/31) ALI, 32.2% (10/31) AKI, and 6.4% (2/31) DIC. Twenty-six (83.9%) patients received enoxaparin for most of their time in hospital (17 on prophylactic and 9 on therapeutic dose) whereas 5 (16.1%) did not; a significant difference in survival between these two groups was observed by Kaplan Meier analysis (Mantel-Cox log rank test p=0.022) (**Figure 1**). Univariate analysis showed the number of patients started on enoxaparin treatment during their hospital stay was higher in the group who died than in surviving patients (p=0.01) (**Table IV**, **panel A**); moreover, enoxaparin was administered more frequently in patients with moderate-severe disease requiring sub-intensive or intensive care than in patients managed on a general care ward (p<0.001) (**Table IV**, **panel B**). In both situations, there is no meaningful difference between the enoxaparin dose regimens used (**Table IV**, **panels A** and **B**).

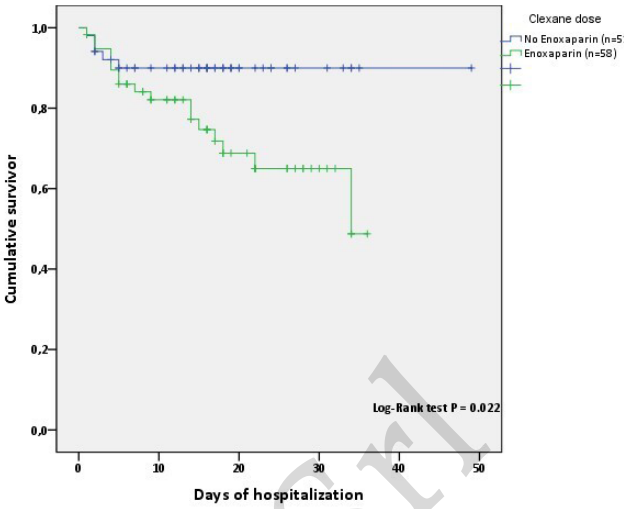


Figure 1 - Kaplan-Meier survival curve of patients who were treated with any dose of enoxaparin and those were not

At univariate analysis, mortality was significantly associated with age, bleeding events, one or more comorbidities, lowe GFR (<30 mL/min), high total bilirubin

Table IV - *Relationship between anticoagulant therapy with enoxaparin and mortality (Panel A) and type of care during hospitalisation (Panel B) of the 141 patients with COVID-19*

Anticoagulant therapy	Mortality		Univariate analysis		
	No	Yes	Odds Ratio	(95% CI)	^p-value
	PANEL A				
Any dose of enoxaparin **No (51)** **Yes (90)**	 46 64	 5 26	3.73	(1.33-10.4)	0.010
Enoxaparin dose **Prophylactic (65)** **Therapeutic (25)**	 48 16	 17 9	1.58	(0.59-4.25)	0.438
	PANEL B				
Any dose of enoxaparin **No (51)** **Yes (90)**	 49 55	 2 35	15.5	(3.56-68.2)	<0.001
Enoxaparin dose **Prophylactic (65)** **Therapeutic (25)**	 41 14	 24 11	1.34	(0.52-3.42)	0.631

^p-value was generated by Fisher's exact test.

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Table V - *Characteristics of the study patients by mortality and results of multivariate regression analysis*

Characteristics	Exitus		Univariate analysis			Logistic regression		
	No	Yes	Odds Ratio	(95% CI)	p-value	Odds Ratio	(95% CI)	p-value
Age **>59.7 years** **≤59.7years**	61 49	8 23	3.57	(1.47-8.69)	0.004	1.19	(0.36-3.92)	0.774
Sex **Male** **Female**	77 33	27 4	2.89	(0.93-8.92)	0.066			
Bleeding events **No** **Yes**	109 1	27 4	16.1	(1.73-150)	0.008	2.82	(0.23-33.9)	0.416
Thromboembolic events **No** **Yes**	100 10	27 4	1.48	(0.43-5.09)	0.509			
Comorbidities **No** **Yes**	38 72	3 28	4.92	(1.40-17.2)	0.007	3.81	(0.70-20.7)	0.121
eGFR value **≥ 30 ml/min** **<30 ml/min**	106 4	24 7	7.72	(2.09-28.5)	0.002	5.90	(1.21-28.7)	0.028
ALT value **≥ 3 ULN** **< 3 ULN**	81 29	24 7	1.22	(0.47-3.15)	0.817			
Total bilirubin **≥ 2 mg/dL** **< 2 mg/dL**	1 109	8 23	37.9	(4.51-318)	<0.001	22.5	(2.15-236)	0.009
Fasting glucose serum levels **≥ 126 mg/dL** **< 126 mg/dL**	29 81	23 8	8.03	(3.23-19.9)	<0.001	3.83	(1.32-11.1)	0.013
Enoxaparin therapy **No** **Yes**	46 64	5 26	3.73	(1.33-10.4)	0.010	2.46	(0.71-8.48)	0.154
Dose of enoxaparin **Prophylactic** **Therapeutic**	48 16	17 9	1.58	(0.59-4.25)	0.438			

eGFR: estimated Glomerular Filtration Rate; ALT: alanine aminotransferase; ULN: upper limit of normal. ^p-value was generated by Fisher's exact test.

(>2 mg/dL), fasting hyperglycemia at diabetic level (>126 mg/dL), and enoxaparin therapy (**Table V**). However, at multivariate logistic regression analysis, only low eGFR, high total bilirubin, and fasting hyperglycemia were independent factors associated with a higher in-hospital mortality (OR 5.9, 95%CI: 1.21-28.7, p=0.028; OR 22.5, 95%CI: 2.15-2.36, p=0.009; OR 3.83, 95% CI: 1.32-11.1, p=0.013, respectively).

DISCUSSION

In this study, among 141 patients with COVID-19 admitted to Cotugno Hospital, the incidence of thrombosis was 9.9%. This percentage is in line with the data published in the literature that reports rates between 4.8-85%[9,12,19].

It should be emphasised that the indication to give a prophylactic dose of heparin to all patients with COVID-19 has been consolidated[20]. Our data refer to the first wave of pandemic, in which approaches about thromboprophylaxis were neither shared or homogeneous; as a general rule, thromboprophylaxis was prescribed in patients with more severe disease and a higher perceived thrombotic risk. Moreover, the incidence of thrombotic events we calculated could also be underestimated because echo-color doppler and screening for PE were not

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systematically performed, since CT angiography was carried out only in cases of desaturation with associated altered coagulation parameters.

We did not observe any significant difference in the incidence of thromboembolic events between patients who received any dose of enoxaparin and those who did not. Specifically, in the evaluation of the clinical cases, we observed that patients who took enoxaparin at prophylactic dose had a lower incidence of thrombosis than patients who received a therapeutic dose.

However, it should be highlighted that 5 of the 6 patients who were diagnosed with PE while receiving therapeutic dose of enoxaparin, had not been taking anticoagulants just before this diagnosis. In these 6 patients, therapeutic dose of enoxaparin was started because of remarkable increases in D-dimer and was confirmed when the CT scan showed PE.

On the whole, these specific clinical courses, the approach to use enoxaparin in more severe/higher-risk patients, and the possible underestimation of thrombotic events suggest a protective role of LMWH, despite the non-significant differences found between those patients receiving and those not receiving thromboprophylaxis.

Although increased D-dimer is now a useful tool to suggest PE, the diagnosis should only be driven by CTPA lung scan, as recently suggested by the ISTH[20,21].

It is noteworthy that in almost all PE there was an involvement of segmental or subsegmental branches of pulmonary arteries. This finding agrees with reports in the literature regarding the increased incidence of PE involving segmental and subsegmental branches[22].

The incidence of haemorrhagic events was 3.5% (5 patients), in all cases major bleeding episodes, without any minor bleeding. Of these 5 patients, 4 received therapeutic dose of enoxaparin and one was not on anticoagulant therapy. Bleeding was associated with a severe clinical course and prognosis, as 3/5 patients required sub-intensive or intensive care during hospitalisation and 4 died.

Our analysis shows that enoxaparin at prophylaxis doses of 40 mg or 60 mg OD is safe, and is not associated with bleeding risk. In our cohort, therapeutic doses of enoxaparin increased the haemorrhagic risk.

Thirty-one patients (22%) died during follow-up. Mortality was higher in patients with 2 or more comorbidities at admission and in those admitted to an ICU. Independent risk factors for mortality were renal damage, and chronic respiratory and liver disease.

On multivariate analysis, we found that age over 59 years, CKD with eGFR <30 mL/min, diabetes mellitus and hyperbilirubinaemia were independently associated with mortality. Therefore, in our study, we did not observe any advantages of enoxaparin, either as prophylaxis or at high doses, in terms of mortality. In particular, even in patients with more severe clinical conditions that required increased heparin use and intensive/sub-intensive care, enoxaparin does not appear to be an independent risk factor for mortality. Recently, the COVID UPO Clinical Team study noted that the use of a prophylactic dosage of enoxaparin appears to be associated with similar in-hospital overall mortality compared to higher doses[23].

In addition, as reported by Pesavento *et al.*[13], high doses of enoxaparin are associated with significant bleeding risk.

In both these studies, the advantage of using enoxaparin at prophylactic doses is confirmed both in terms of prevention of thrombotic events and in terms of safety; in our study no patient on prophylaxis experienced a bleeding event.

CONCLUSIONS

In spite of the limitations of its retrospective design and the heterogeneous treatment approaches, our study may provide information about the efficacy and safety of prophylactic doses of enoxaparin in hospitalised patients from both general care wards, and sub-ICUs and ICUs. Rigorous controlled international and national clinical trials are currently ongoing and their results are awaited. These will help establish the efficacy and safety of enoxaparin at therapeutic doses and the possible advantages compared with prophylactic doses in terms of mortality and general clinical outcomes of hospitalised COVID-19 patients.

AUTHORSHIP CONTRIBUTIONS

RA designed the study, interpreted the data and critically revised the paper. MV and MPU collected and interpreted the data and wrote the paper; SP, AS, LLF, FB, LA, FS, NM, FF, SS, RP, GF collected the data. DI analysed the data. RZ reviewed the manuscript. All Authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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The Authors declare no conflicts of interest.

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