THROMBOTIC DISORDERS, PROTHROMBOTIC ABNORMALITIES AND COVID-19

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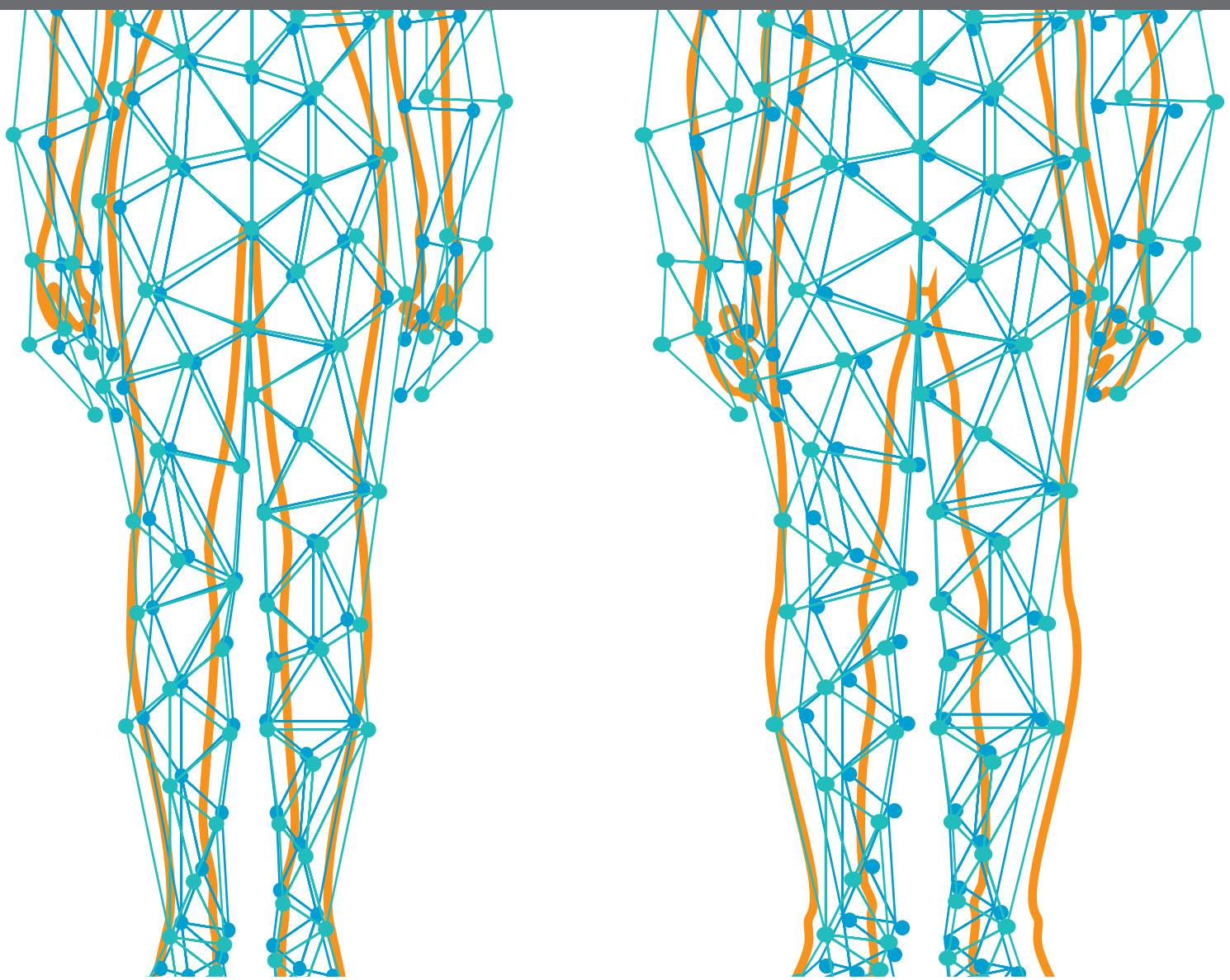

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THROMBOTIC DISORDERS, PROTHROMBOTIC ABNORMALITIES AND COVID-19

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EDITORIAL
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Editorial: Thrombotic Disorders, Prothrombotic Abnormalities and COVID-19

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Keywords: COVID-19, thrombosis, pulmonary embolism, immune thrombocytopenia, stroke, metformin, d-dimer, deep vein thrombosis

Editorial on the Research Topic

Thrombotic Disorders, Prothrombotic Abnormalities and COVID-19

Hypercoagulability is one of the major hallmarks of the COVID-19 course, manifesting as venous and arterial thromboembolism along with cardiovascular and lung failure. While biomarkers, such as a persistent increase in D-dimer and troponin levels, have been suggested to reflect disease severity, improved risk assessment is vital for the optimal anticoagulant management of this life-threatening condition.

In this collection, authors from different countries confirm that the prothrombotic conditions characterized by increased d-dimer and troponin are present in inpatients with COVID-19 (Goudot et al. and Tassiopoulos et al.) and this increase may also influence the approach to thromboprophylaxis in this clinical setting. Regarding venous thromboembolism prevention inpatients next to orthopedic surgery, in fact, no differences with traditional thrombphylactic doses of low molecular weight heparin approach has been found in a study (Perazzo et al.), while inpatients hospitalized for COVID-19 in regular clinic ward without surgical urgencies or intensive care supports may benefit of increased prophylactic doses of enoxaparin or regular doses of fondaparinux (Russo et al.).

Yet, in the daily clinical practice, based on extended prophylactic anticoagulation, the best way to detect venous thromboembolism with objective methods remains a matter of debate, and the optimal timing of ultrasound scan or lung CT scan are a part of this problem (Lapébie et al.). Some authors recommend to perform radiological diagnostic evaluation at the disease onset, while others propose such evaluation if d-dimer levels are increasing, considering hospitalization for COVID-19 an additional confounding factor to thrombosis development. A second diagnostic assessment for thrombotic disorders may be useful prior to modification of thomboprophylactic regimen. Clinical overt pulmonary embolism and/or fatal pulmonary embolism may occur, in fact, not only in critically ill patients (Benito et al.) in ICU but also in patients in a non-intensive ward during the hospitalization (Benito et al. and Gratz et al.).

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In addition, the occurrence of atherothrombotic diseases is not infrequent in COVID-19 (Sattar et al.). Cardiovascular risk factors such as hypertension, obesity, and diabetes may trigger arterial thrombotic events in this clinical setting in the presence of prothrombotic gene polymorphism associated with atherthrombosis, like ACE insertion/deletion polymorphism (Calabrese et al.). Hence, several drugs have been suggested to modulate the atherothrombotic risk with metformin being one of most efficacious in this setting (Al-Kuraishy et al.).

Autoimmune diseases modifying the clotting balance have been reported as a late complication of COVID-19. From a pathophysiological point of view, the cytokine storm with imbalanced autoimmunity has been identified as a main player in these clinical conditions (Guo et al.). In this setting, also COVID-19-associated immune thrombocytopenia may occur (Hindilerden et al.) and a differential diagnosis with heparin induced thrombocytopenia should be performed because it may further complicate this challenging clinical scenario, with possible aggravation of thrombosis and disseminated intravascular coagulation.

The extreme clinical variability of the novel pandemic disease requires robust advances in diagnostics and management strategies. This is vital, since the COVID-19 pandemic is ongoing, while vaccination is still in its early stages.

AUTHOR CONTRIBUTIONS

PD planned the editorial. MN identified references. AR and BB performed revision of text. All authors contributed to the article and approved the submitted version.

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Guest Editors like to thank all the scientific community that showed interest with submission to this collection. We have been honored to learn clinical experience from all of them.

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CASE REPORT
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Immune Thrombocytopenia in a Very Elderly Patient With Covid-19

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Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by a decreased number of platelets and mucocutaneous bleeding. Many viruses have been identified as triggers of the autoimmune process, including human immunodeficiency virus (HIV), hepatitis C virus (HCV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus, rubella, and measles. Association with the new severe acute respiratory syndrome coronavirus, SARS-CoV-2 infection (Covid-19 infection) has been rarely reported. Here, we report the oldest case of ITP patient triggered by the novel coronavirus infection. He showed inadequate response to IVIG but responded to corticosteroids with no severe adverse events. Further studies are warranted to determine the optimal therapeutic strategies for ITP with the Covid-19 infection.

Keywords: immune thrombocytopenia, Covid-19, very old age, intravenous immunoglobulin, corticosteroids

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INTRODUCTION

Immune thrombocytopenia (ITP) is a rare autoantibody-mediated disorder characterized by a platelet count of $<100{,}000/mm^3$, mostly with minor mucosal bleeding (1). ITP occurs either *de novo* or secondary to other underlying disorders. Common conditions associated with secondary ITP include lymphoproliferative disorders, other autoimmune disorders and collagen vascular diseases. ITP is also associated with certain, mostly viral, infections (2). HIV and HCV are well-characterized causes of ITP while Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes viruses, parvovirus, rubella, and measles have also been identified as causes of ITP (2). An ongoing outbreak of SARS-CoV-2 infection (Covid-19 infection) was first identified in Wuhan, Hubei province, China in December 2019 (3). Thrombocytopenia is a risk factor for increased morbidity and mortality in patients with Covid-19 infection (4). Thrombocytopenia in Covid-19 patients may be the result of disseminated intravascular coagulation (DIC), sepsis or may be drug-induced (4). ITP in Covid-19 has been rarely reported (5, 6). We report a 86-year-old male diagnosed with Covid-19 infection, who at initial diagnosis of Covid-19 infection presented with severe ITP.

CASE PRESENTATION

A 86-year-old man with a history of hypertension and type 2 diabetes presented with a 1-week history of excessive bruising, fatigue, fever, and dry cough. He had known Covid-19 exposure. On physical examination, he was subfebrile (37.6°C) and had a respiratory rate of 24/min. There were purpuric eruptions widely scattered over the skin and hemorrhagic bullae in the oral cavity (**Figure 1**). The patient's clinical signs included hypoxaemia (pulse oximetry 91% on ambient air) and sinus tachycardia (110 beats/minute). Lung auscultation revealed diminished breath

sounds with fine bibasilar crackles. The laboratory tests showed the following: hemoglobin 11 g/dL, total leukocyte count 4,020/mm^3, neutrophil 2,930/mm^3, lymphocyte: 960/mm^3, and platelet count 10,000/mm^3 (**Table 1**). On biochemical tests, C-reactive protein was elevated at 15 mg/L (normal range, 0–5) with normal procalcitonin level (0.07 ng/ml; normal range < 0.5). Serum ferritin, LDH, and Troponin-I levels were normal (65 μg/ml, 247 U/L, and 10 pg/ml, respectively). Prothrombin and activated partial thromboplastin time were normal. Fibrinogen level was 379 mg/dl (normal range, 200–400) and D-Dimer was slightly elevated (0.97 μg/ml; normal range, 0–0.5) (**Table 1**). Reverse transcriptase PCR assay detected the presence of SARS-CoV-2 RNA in the nasopharyngeal swab. Chest computed tomography (CT) showed widespread scattered ground-glass opacities in both lungs, findings compatible with severe Covid-19 pneumonia (**Figure 2**). On peripheral blood smear, there were no schistocytes or atypical cells. Peripheral blood confirmed the presence of thrombocytopenia. Given the very old age of the patient and a possible association with multiple myeloma (MM) and ITP, immunoglobulin levels, serum and urine immunofixation and protein electrophoresis were checked and all were found to be within normal ranges (7). Bone marrow aspiration revealed a normocellular bone marrow with concomitant increase in normal sized megakaryocytes. The other cell lines were normal and there was no sign of dysplasia and hemophagocytosis. Bone marrow biopsy also revealed increased number of megakaryocytes in the absence of other significant abnormalities. The cytogenetic analysis revealed normal karyotype. To treat Covid-19 pneumonia, favirapivir 1,600 mg twice daily on day 1, followed by 600 mg twice daily for a total duration of 5 days and azithromycin 500 mg on day 1 plus 250 mg daily on days 2–5 were started. It was considered that the patient developed secondary ITP triggered by Covid-19. Other viral, autoimmune and malignant diseases were screened and found to be negative. Due to the presence of hemorrhagic bullae, the patient was regarded to have high risk for life threatening bleeding due to secondary ITP. Intravenous immunoglobulin (IVIG) was administered at a rate of 1 g/kg body weight for two consecutive days. Three days after the initiation of IVIG, his platelet count was 25,000/mm^3. Thus, oral prednisolone at a dose of 1 mg/kg/day was started. On the 10th day of admission to hospital, the purpura had disappeared and his oxygen saturation on ambient air was 96%. His platelet count increased to 100,000/mm^3. Due to the hematological response attained on the 7th day of prednisolone and taking into consideration the side effects of corticosteroids related to his comorbidities and very old age, the dose was decreased to 0.5 gr/kg/day and planned to be stopped in 4 weeks. He is now on the 3th week of the corticosteroid treatment at a dosage of 0.25 gr/kg/day with no bleeding symptoms. The final laboratory tests showed the following: hemoglobin 11 g/dL, total leukocyte count 4,200/mm^3, neutrophil 2,400/mm^3, lymphocyte: 1,680/mm^3 and platelet count 150,000/mm^3.

DISCUSSION

Covid-19 is a systemic infection with significant impact on the hematopoietic system. On admission, 36.2% of patients present with thrombocytopenia, which is more prominent among severe vs. non-severe cases (57.7 vs. 31.6%) (8). Thrombocytopenia is a risk factor for increased morbidity and mortality in Covid-19 infection %) (4). Thrombocytopenia in Covid-19 patients may be caused by sepsis, disseminated intravascular coagulation (DIC), or drug-induced (4). Recently, several case reports have suggested that ITP may be associated with Covid-19 infection (5, 6). ITP is a rare autoimmune disease, in which with many viruses including mainly HIV and HCV have been identified as triggers of the autoimmune process (2).

The mechanism of virus-induced thrombocytopenia has not been clearly elucidated. Viruses may cause a decrease in platelet production by infecting megakaryocytes. This results in apoptosis of megakaryocytes, decreased maturation of megakaryocytes or decreased expression of the thrombopoietin receptor. Viruses may also infect hematopoietic stem cells and result in a decrease of progenitor cells and induction of growth deficient megakaryocyte colony forming units, due to disordered production of cytokines by the infected cells in the bone

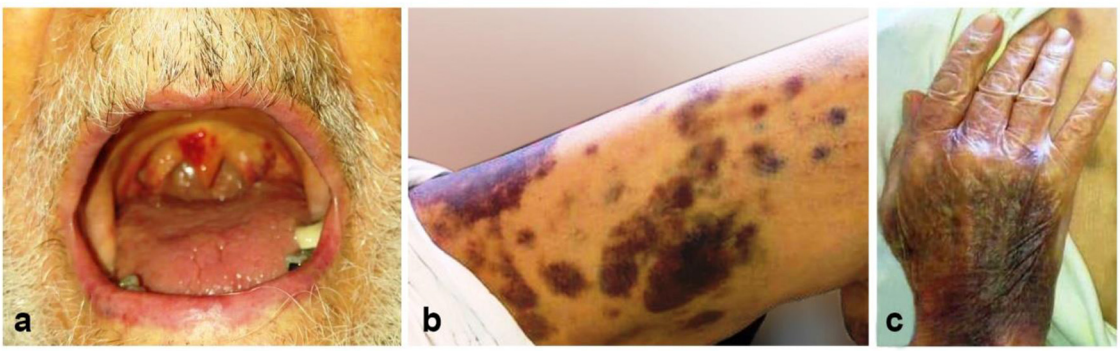

FIGURE 1 | The appearance of the lesions at admission to our center. Hemorrhagic bullous lesions in the oral cavity **(a)**, ecchymotic and purpuric lesions scattered over the lower extremity **(b),** and dorsum of the hand **(c)**.

TABLE 1 | Characteristics of the patient with Covid-19 associated ITP.

	Patient
Age	86
Gender	Male
Hemoglobin level (g/dL)	11
Leukocyte level (/mm^3)	4,020
Lymphocyte level (/mm^3)	960
Platelet count (/mm^3)	10,000
C-reactive protein (mg/L)	15
Procalcitonin level (ng/ml)	0.07
Serum ferritin (μg/ml)	65
LDH level (U/L)	247
Troponin-I (pg/ml)	10
Fibrinogen level(mg/dl)	379
D-dimer(μg/ml)	0.97
Chest CT	Severe pneumonia
Nasopharyngeal swab (tested by PCR)	Positive

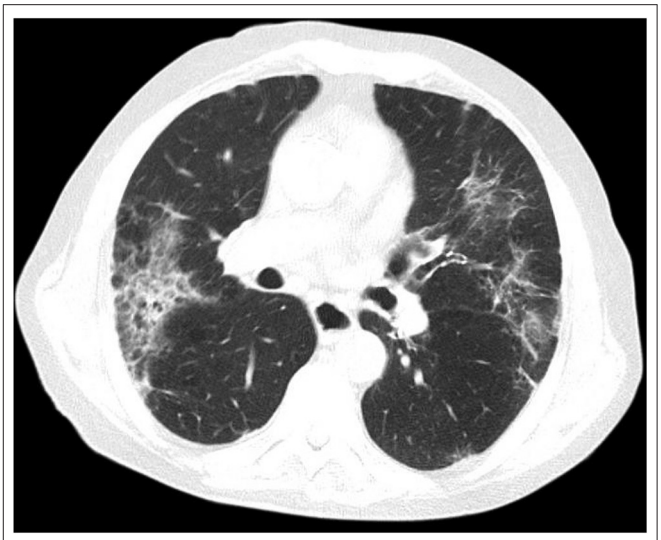

FIGURE 2 | Chest computed tomography shows widespread scattered ground-glass opacities in both lungs, findings compatible with severe Covid-19 pneumonia.

marrow. Another proposal for virus induced thrombocytopenia is by platelet destruction where viruses either directly interact with platelets or recognize immunocomplexes of IgGs and viral antigens (9). Bone marrow examination of our patient showed no suppression of the hematopoietic precursors but an increase in the number of megakaryocytes suggesting that there was immune mediated destruction of platelets. The precise mechanism of ITP associated with the novel coronavirus has not been clearly elucidated. It is presumed that following the infection, the immune responses raised against Covid-19 may cross-react with human proteins that share peptide sequences with the virus and thus result in autoimmune pathologic sequelae (10). Zulfiqar et al. was the first to report ITP in a 65-year-old patient with Covid-19 (5). In that report, ITP developed after cessation of Covid-19 associated symptoms. Bomhof et al. reported a case series of ITP in Covid-19 patients including a 67-year-old man, a 66-year-old woman and a 59-year-old man (6). In the aforementioned case series, ITP developed not only during active COVID-19 infection, but also up to 10 days after the resolution of Covid-19 symptoms. Our patient developed ITP at initial presentation as he was suffering from Covid-19 associated symptoms. First reported case of ITP in the course of Covid-19 responded to prednisolone and eltrombopag (5). While two of the three reported cases recovered from ITP with IVIG and dexamethasone, one patient died of intracerebral bleeding because of delay in diagnosis (6).

To our knowledge, our case is the oldest case of ITP patient triggered by Covid-19 infection. Studies of older ITP adults are lacking, and recommendations for management are based mainly on expert opinion. The treatment of ITP may be difficult, especially in patients older than 75 years (very old age) and must take into account the comorbidities, concurrent medications and severity of bleeding. The mechanism of increased risk of bleeding in older age ITP patients are not at all completely understood, but age is associated with endothelial dysfunction (11). Herein we reported a very old Covid-19 patient presenting with hemorrhagic bullous lesions with high propensity to life threatening bleeding. With prolonged life expectancy, the frequency of ITP has increased and become more challenging in the elderly. Yet, our case implies that IVIG and corticosteroids may remain as optimal first-line treatments in elderly Covid-19 patients presenting with ITP. Eltrombopag is commonly used for treatment of ITP (12). Since eltrombopag, in selected cases, have posed an increased risk for venous thromboembolism, it should be used with caution in Covid-19 infection, which itself is reported to result in a hypercoaguable state (13, 14). Furthermore, there is limited data on the safety of eltrombopag in older ITP patients. The incidence of a thrombotic event was significantly increased in ITP patients ≥65 years undergoing eltrombopag treatment (15). After inadequate response to IVIG, our patient received prednisolone resulting in a platelet count of 100,000/mm^3 on day 10. We refrained from the use of eltrombopag because of the lack of safety data in the elderly ITP patients and to avoid the risk of exacerbation of coagulation activation by Covid-19 infection.

Herein, we reported a case of a 86-year-old male patient with a background history of hypertension, type 2 diabetes and a positive swab for Covid-19, who presented with excessive bruising, fatigue, fever, and dry cough and signs of pneumonia. Our patient had no history of autoimmune disorder. The distinctive feature of our patient is his very old age and that he develops ITP at initial presentation as he was suffering from Covid-19 associated symptoms not after the resolution of Covid-19 symptoms, which contrasts with most of the previous cases reporting ITP after the resolution of Covid-19 symptoms (5, 6). Our findings support that ITP

at initial presentation may be observed in Covid-19 infected patients and other potential causes of thrombocytopenias should be excluded in these patients to avoid lethal complications and deliver appropriate treatment. Given the efficacy of steroids and IVIG based on expert opinion in elderly ITP patients, this therapy is worth considering as a treatment of elderly ITP with the Covid-19 infection. However, taking into account the report by "Centers for Disease Control and Prevention" and "World Health Organization" that states corticosteroids may inhibit immune responses and pathogen clearance of Covid-19, prolonged treatment with steroids should be avoided.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Comittee of Bakirköy Dr. Sadi Konuk Training and Research Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors collected data, wrote, and revised the article.

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ORIGINAL RESEARCH
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Pulmonary Thrombosis or Embolism in a Large Cohort of Hospitalized Patients With Covid-19

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Objective: We set out to analyze the incidence and predictive factors of pulmonary embolism (PE) in hospitalized patients with Covid-19.

Methods: We prospectively collected data from all consecutive patients with laboratory-confirmed Covid-19 admitted to the Hospital de la Santa Creu i Sant Pau, a university hospital in Barcelona, between March 9 and April 15, 2020. Patients with suspected PE, according to standardized guidelines, underwent CT pulmonary angiography (CTPA).

Results: A total of 1,275 patients with Covid-19 were admitted to hospital. CTPA was performed on 76 inpatients, and a diagnosis of PE was made in 32 (2.6% [95%CI 1.7–3.5%]). Patients with PE were older, and they exhibited lower PaO_2:FiO_2 ratios and higher levels of D-dimer and C-reactive protein (CRP). They more often required admission to ICU and mechanical ventilation, and they often had longer hospital stays, although in-hospital mortality was no greater than in patients without PE. High CRP and D-dimer levels at admission ($\geq$150 mg/L and $\geq$1,000 ng/ml, respectively) and a peak D-dimer $\geq$6,000 ng/ml during hospital stay were independent factors associated with PE. Prophylactic low molecular weight heparin did not appear to prevent PE. Increased CRP levels correlated with increased D-dimer levels and both correlated with a lower PaO_2:FiO_2.

Conclusions: The 2.6% incidence of PE in Covid-19 hospitalized patients is clearly high. Higher doses of thromboprophylaxis may be required to prevent PE, particularly in patients at increased risk, such as those with high levels of CRP and D-dimer at admission. These findings should be validated in future studies.

Keywords: COVID-19, pulmonary thrombosis, pulmonary embolism (MeSH), thromboprophylaxis, anticoagulant (MeSH), thromboinflammation

INTRODUCTION

Since December 2019, the rapid spread of the novel betacoronavirus, named SARS-CoV-2, has led to a global pandemic of coronavirus disease 2019 (Covid-19). Several recent studies have shown that patients with Covid-19 frequently have coagulation disorders, especially a marked increase in D-dimer (1–5). These abnormal coagulation parameters have been associated with worse outcomes (1, 2). It is also suggested that Covid-19 predisposes patients to a higher risk of thrombotic disorders, including both venous and arterial thromboembolic disease (3, 4). Several scientific societies and authors have already proposed specific guidelines and recommendations on the use of thromboprophylaxis in patients with Covid-19 (6–11), although the best thromboprophylaxis regimen (dosing and duration), taking into account the characteristics of different groups of patients, has not been established.

To date, a few case series studies (12–19) and some case reports (17, 20–30) have investigated the incidence and features of pulmonary thrombosis or embolism (PE) in patients with Covid-19. All studies included small numbers of cases, and most of them were conducted in ICU patients (12, 16–19), as they constitute a particular group with an increased risk of PE (14). Other studies have been based on patients who underwent computed tomography pulmonary arteriography (CTPA) but did not provide clear information on the baseline population (14, 15). These limitations make it difficult to know the true incidence, characteristics, and risk factors of PE in patients with Covid-19.

Using a large cohort of consecutive patients with laboratory-confirmed Covid-19 admitted to a single university hospital, we sought to analyze the incidence, clinical features, and predictive factors of PE in patients with Covid-19.

METHODS

Setting

We conducted this study at the Hospital de la Santa Creu i Sant Pau, a tertiary acute care university hospital in Barcelona, Spain. The hospital's Institutional Review Board approved the study.

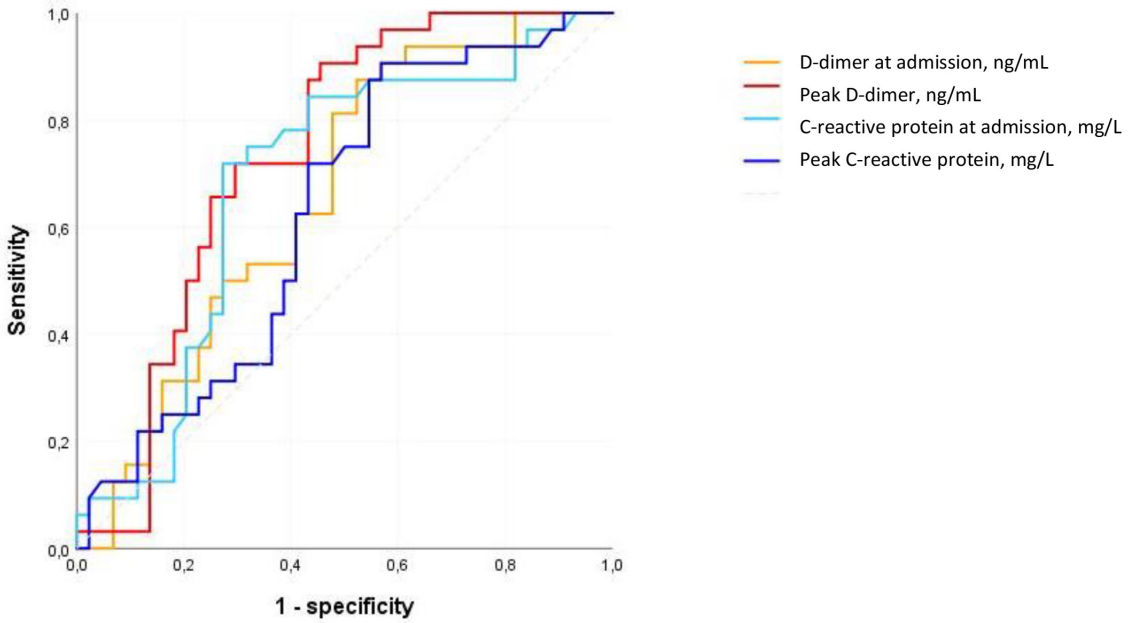
We collected data prospectively from all consecutive patients with laboratory-confirmed Covid-19 diagnosed at our hospital between March 9 and April 15, 2020, and we included those who were admitted to hospital.

Specific guidance based on the International Society of Thrombosis and Hemostasis interim guidelines (6) was developed at our center and distributed on March 18 to all attending staff. It was recommended to start prophylactic doses of subcutaneous low molecular weight heparin (LMWH) (enoxaparin 4,000 IU/24 h, bemiparin 3,500 IU/24 h or tinzaparin 4,500 IU/24 h) for all patients (including the non-critically ill) who required hospital admission for Covid-19 in the absence of contraindications. Doses were adjusted to body weight. On April 3, 2020, the guidance was subsequently amplified by adding the use of high-dose prophylaxis for patients at increased thrombotic risk, including those with D-dimer >3,000 ng/mL (enoxaparin 100 IU/kg/ 24 h, bemiparin 75–80 IU/kg/24 h, or tinzaparin 100 IU/Kg/ 24 h).

TABLE 1 | Characteristics of patients with Covid-19 with and without pulmonary embolism.

Variable	Pulmonary embolism *N* = 32	NO pulmonary embolism *N* = 44	*P*-value
Age, years—median (IQR)	66 (13)	60 (17)	0.110
Age ≥ 60 years—no. (%)	24 (75)	22 (50)	**0.028**
Male gender—no. (%)	20 (62.5)	31 (70.5)	0.466
Diabetes mellitus—no. (%)	6 (18.8)	6 (13.6)	0.546
Hypertension—no. (%)	15 (46.9)	19 (43.2)	0.749
Chronic lung disease—no. (%)	4 (12.5)	12 (27.3)	0.119
Active cancer—no. (%)	5 (15.6)	2 (4.5)	0.124
BMI—median (IQR)	28.1 (5.4)	26.7 (8)	0.527
BMI ≥ 25—no. (%)	22 (75.9)	27 (71.1)	0.660
Obesity—no. (%)	8 (27.6)	13 (32.5)	0.661
Area of admission in the hospital at the time of performing CTPA—no. (%)			0.938
- emergency department	4 (12.5)	6 (13.6)	
- inpatient ward	23 (71.9)	30 (68.2)	
- intensive care unit	5 (15.6)	8 (18.2)	
Days from admission until CTPA performed—median (IQR)	7 (10.5)	5 (10)	0.398
LMWH administration before CTPA—no. (%)	28 (87.5)	39 (88.6)	1
Days of LMWH administration before CTPA—median (IQR)	6 (9)	5 (9)	0.922
LMWH doses—no. (%)			0.823
- prophylactic doses	26 (92.9)	34 (87.2)	
- "higher risk" prophylactic doses	2 (7.1)	4 (10.3)	
- therapeutic doses	0 (0)	1 (2.6)	
Pneumonia in the CTPA—no. (%)	31 (96.9)	41 (93.2)	0.634
PaO_2:FiO_2 at the time of performing CTPA	222 (163.5)	250 (181.8)	0.102
Invasive mechanical ventilation at the time of CTPA—no. (%)	7 (21.9)	6 (13.6)	0.346
Worst PaO_2:FiO_2–median (IQR)	158.5 (134.8)	228.5 (170.5)	**0.017**
Patients needing ICU admission during their hospital stay—no. (%)	15 (46.9)	10 (22.7)	**0.027**
Patients requiring invasive mechanical ventilation during their hospital stay—no. (%)	14 (43.8)	8 (18.2)	**0.015**
D-dimer at admission, ng/mL—median (IQR)	5,274.5 (16,419)	1,045.5 (6,287.8)	**0.017**
D-dimer at admission ≥ 1,000 ng/mL—no. (%)	26 (81.3)	23 (52.3)	**0.009**
Peak D-dimer, ng/mL—median (IQR)	22,791 (42,552)	6039.5 (15,982.3)	**0.001**
Peak D-dimer ≥ 3,000 ng/mL—no. (%)	31 (96.9)	29 (65.9)	**0.001**
Peak D-dimer ≥ 6,000 ng/mL—no. (%)	29 (90.6)	23 (52.3)	**<0.001**
C-reactive protein at admission, mg/L—median (IQR)	161.5 (65)	83.5 (126)	**0.007**
C-reactive protein at admission ≥ 150 mg/L—no. (%)	24 (75)	14 (31.8)	**<0.001**
Peak C-reactive protein, mg/L—median (IQR)	221.5 (169.4)	183 (236.4)	0.059
Hospital stay, days—median (IQR)	15.5 (9.8)	7 (11)	**0.010**
In-hospital death—no. (%)	3 (9.4)	5 (11.4)	1

BMI, body mass index; CTPA, computed tomography pulmonary arteriography; FiO_2, Fraction of inspired oxygen; IQR, interquartile range; LMWH, low molecular weight heparin; PaO_2 partial pressure of arterial oxygen.
Bold values refer to P-values that are statistically significant (< 0.05); the idea is to make easer the identification of statistically significant variables in this large table.



	AUC (95% confidence interval)	p-value
D-dimer at admission, ng/mL	0.661 (0.539–0.782)	0.017
Peak D-dimer, mg/mL	0.734 (0.621–0.848)	0.001
C-reactive protein at admission, mg/L	0.684 (0.560–0.808)	0.007
Peak C-reactive protein, mg/L	0.627 (0.501–0.753)	0.059

FIGURE 1 | Receiver operating characteristic (ROC) curve for D-dimer and C-reactive protein as predictors of pulmonary embolism in patients with Covid-19. AUC, area under the ROC curve.

Hospital guidance on when to suspect possible PE and request a CTPA in Covid-19 patients was also developed and distributed to all attending staff. Suspicion of having PE included those patients whose partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2:FiO_2) ratio worsened or failed to improve, associated with an increasing or persistently high D-dimer (>3,000 ng/mL) and/or hemodynamic deterioration or other "classic" symptoms of PE, such as pleuritic chest pain, hemoptysis, syncope, and/or signs of right ventricular strain. Patients with suspicion of PE had a CTPA and started anticoagulant therapy with full-dose LMWH if PE was diagnosed.

All CTPA scans were performed using a 16-slice multi-detector CT (Philips Brilliance CT 16 C Slice) after intravenous injection of 60 ml iodinated contrast agent (Optiray Ultraject 350 mg/mL Ioversol, Guerbet, France) at a flow rate of 4 mL/s, triggered on the main pulmonary artery. The CT scan settings were 120 kVp, slice thickness 2 mm, increment 1 mm, pitch 0.688, rotation time 0.28 s, and average tube current 300 mA. The location of embolus (main pulmonary, lobar, segmental, and subsegmental artery) and clot burden (low, moderate, and high) according to a modified Qanadli Score (31) were evaluated. Right ventricle overload was also assessed (right ventricle diameter to left ventricle diameter ratio >1.3). Following the clinical guidelines developed at our medical center and the recommendations of the Spanish Agency for Medicinal Products and Medical Devices (AEMPS), a single dose of intravenous tocilizumab (600 mg for patients ≥75 kg; 400 mg for those <75 kg) was suggested as treatment for hospitalized patients with Covid-19 and data for cytokine release syndrome.

Variables Assessed

We collected information on patients who underwent CTPA for suspected PE. Variables included demographic data, preexisting chronic medical conditions, body mass index, thrombosis prophylaxis with LMWH and dosing, diagnosis and characteristics of PE, PaO_2:FiO_2 ratio, estimated using methods developed by Brown and colleagues (32, 33) (PaO_2:FiO_2 at the time of CTPA and worst ratio during hospital stay), evolution of D-dimer and C-reactive protein (at admission and peak level), and

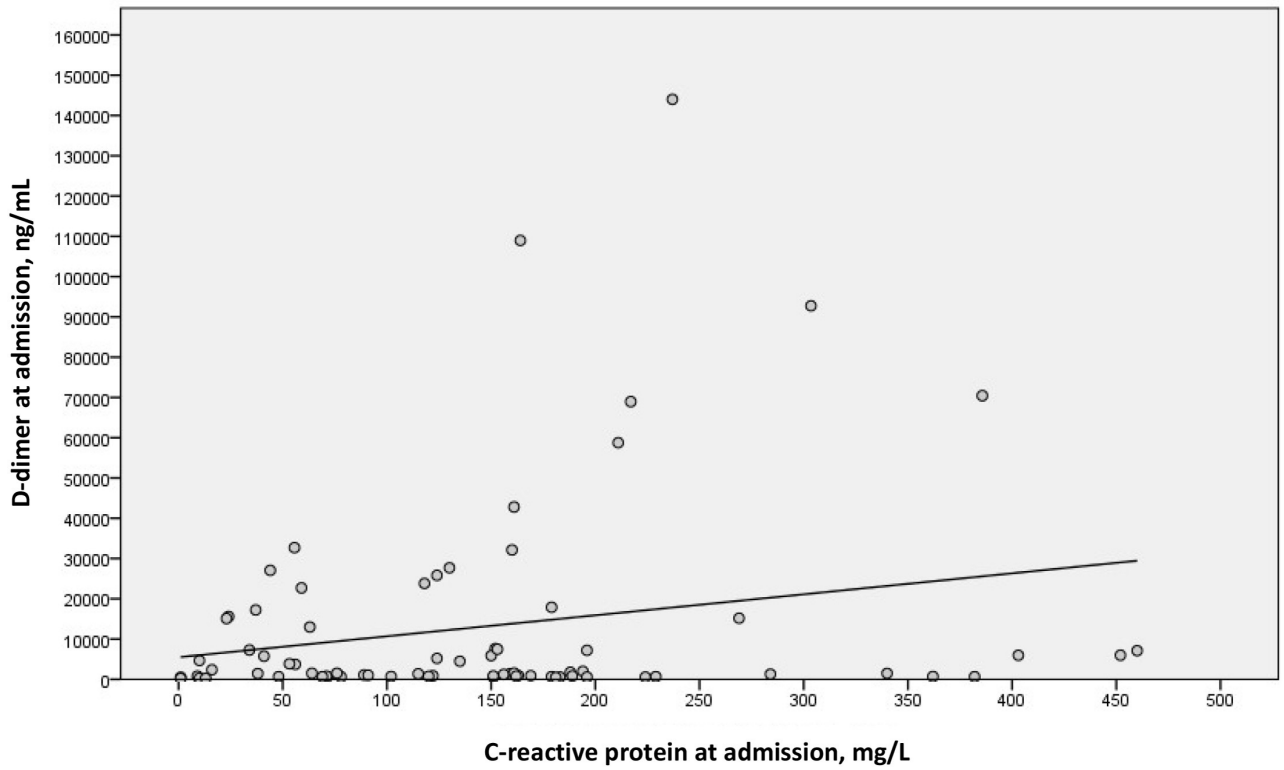


FIGURE 2 | Correlation between D-dimer and C-reactive protein at admission in patients with Covid-19 and suspected pulmonary embolism ($r_s = 0.146$; $p = 0.208$).

clinical outcomes, including need for invasive mechanical ventilation, ICU admission, length of hospital stay, and in-hospital death.

Statistical Analysis

We summarized continuous variables as medians and interquartile ranges and categorical variables as percentages of the total sample for that variable. The incidence of PE was estimated with a 95% confidence interval (CI). We used the Wilcoxon rank-sum and chi-square tests (or Fisher's exact tests when appropriate) to evaluate group differences (Covid-19 patients with and without PE in the CTPA) for continuous and categorical variables, respectively. A multivariable logistic regression model was used to identify factors independently associated with a higher risk of developing PE. Any variable tested in univariate analysis with a $p < 0.25$, together with all variables of known clinical importance, were selected as candidates for the first multivariate model. We then followed the purposeful selection of covariates method described by Hosmer et al. (34) Final parameter estimates are shown as odds ratios (OR) with their corresponding 95% CIs. Correlations between quantitative variables were examined using the Spearman rank correlation test. $P < 0.05$ were considered to be significant for all statistical tests. Data were analyzed using IBM® SPSS®, version 26.0.

RESULTS

Of 1,863 consecutive patients diagnosed with laboratory-confirmed Covid-19 between March 9 and April 15, 2020, at our center, a total of 1,275 patients (68.4%) were admitted to hospital. A total of 146 inpatients (12.4%; 95% CI 10.5–14.4%) died during their hospital stay [101 patients (7.9%) were still hospitalized at the time of data analysis].

During this period, a CTPA was indicated for suspicion of PE in 76 inpatients (6% of patients with Covid-19 admitted to hospital). CTPA confirmed a diagnosis of PE in 32 patients (42.1% of tests), which represents a cumulative incidence of 2.6% (95 CI 1.7–3.5%) for PE among Covid-19 inpatients. Most CTPAs were requested for patients admitted to conventional wards (70%); in fact, a similar percentage of patients with (71.9%) and without PE (68.2%) were hospitalized in the wards at the time of diagnosis (**Table 1**).

Table 1 shows the characteristics of Covid-19 inpatients with and without PE in the CTPA. Patients with PE were more often ≥60 years old, had lower PaO_2:FiO_2 ratios, and had higher levels of D-dimer and C-reactive protein. The D-dimer levels of these patients were therefore more than five times higher at hospital admission, and the peak D-dimer level during hospital stay was more than three times higher than in patients without PE. Moreover, C-reactive protein at admission was almost twice as high among PE patients. Covid-19 inpatients with PE more often required ICU admission

and invasive mechanical ventilation than those without PE and had longer hospital stays, although in-hospital mortality was not statistically significantly different between the two groups (9.4% vs. 11.4%). There were also no significant differences between in-hospital mortality in PE patients and mortality in all admitted Covid-19 patients ($p = 0.604$). A high proportion of patients with and without PE (87.5% and 88.6, respectively) were receiving LMWH prior to CTPA, mostly at prophylactic doses, with no statistically significant differences between the two groups.

We did not investigate deep vein thrombosis (DVT), except in symptomatic patients. We found DVT in two patients with PE, one of whom was a patient with gastric cancer.

Candidate variables for the first multivariable model were: age $\geq$ 60 years, sex, chronic lung disease, active cancer, BMI, LMWH administration, D-dimer at admission $\geq$ 1,000 ng/mL, C-reactive protein at admission $\geq$ 150 mg/L, ICU admission, worst PaO_2:FiO_2, and peak D-dimer $\geq$ 6,000. Multivariate analysis found the following independent factors as associated with an increased risk of having PE: C-reactive protein at admission $\geq$ 150 mg/L (OR 7.9, 95% CI 2.4–26.7), D-dimer at admission $\geq$ 1,000 ng/mL (OR 4.5, 95% CI 1.2–17.2, $p = 0.026$), and peak D-dimer $\geq$ 6,000 ng/mL during hospital stay (OR 5.6, 95% CI 1.3–24.5) (Nagelkerke $R^2 = 0.439$).

We analyzed D-dimer and C-reactive protein as predictors of PE using ROC curves. The peak D-dimer during hospital stay followed by C-reactive protein at admission had the largest area under the ROC curve (**Figure 1**).

We examined a possible linear correlation between levels of D-dimer and C-reactive protein at admission (**Figure 2**) and at their highest levels during hospital stay (**Figure 3**). A positive linear correlation was observed in both cases and was statistically significant for peak D-dimer and peak C-reactive protein values during hospitalization. We also found statistically significant negative correlations between peak D-dimer levels and worst PaO_2:FiO_2 ratio (**Figure 4**) and between peak C-reactive protein levels and worst PaO_2:FiO_2 ratio (**Figure 5**) during hospital stay.

In patients with PE, the CTPA showed mainly affection of the segmental and subsegmental branches of pulmonary arteries, predominantly with a low thrombus load (**Table 2**).

DISCUSSION

In this analysis involving a large sample of consecutive patients hospitalized with Covid-19, we found a high incidence of PE of 2.6% despite the wide use of thromboprophylaxis. Patients with PE were older and had lower PaO_2:FiO_2 ratios and markedly higher levels of D-dimer and C-reactive protein. They more often required admission to ICU, invasive mechanical ventilation, and longer hospital stays, although in-hospital mortality was no greater than in patients without PE. We identified high C-reactive protein and D-dimer at admission (≥150 mg/L and

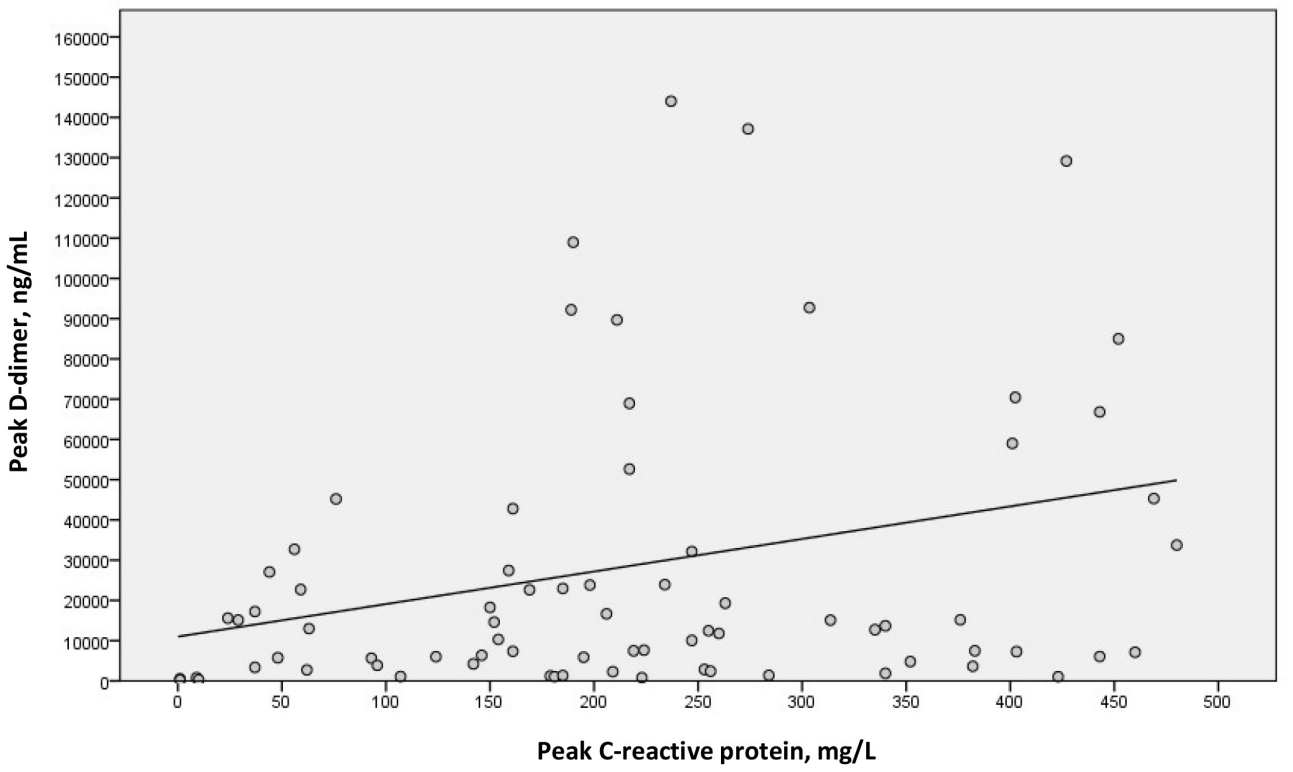


FIGURE 3 | Correlation between peak D-dimer and peak C-reactive protein during hospital stay in patients with Covid-19 and suspected pulmonary embolism ($r_s = 0.279$; $p = 0.015$).

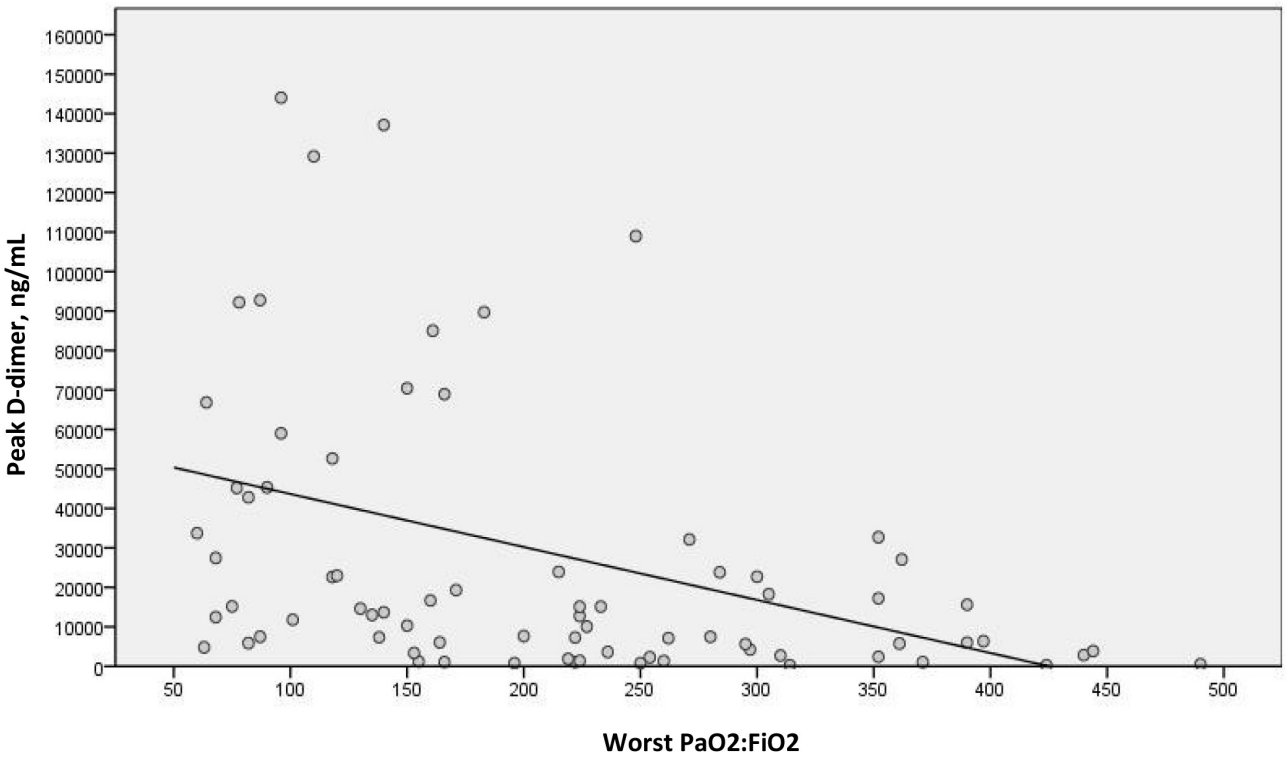


FIGURE 4 | Correlation between peak D-dimer and worst PaO_2:FiO_2 during hospital stay in patients with COVID-19 and suspected pulmonary embolism ($r_s = -0.471$; $p < 0.001$).

≥1,000 ng/ml, respectively) and a peak D-dimer during hospital stay ≥6,000 ng/ml as independent factors associated with PE. Prophylactic doses of LMWH did not, however, appear to prevent PE.

Most of the studies on PE in patients with Covid-19 have been conducted in ICU patients, who are at greater risk of venous thromboembolism and have shown incidences ranging from 17 to 35% (12, 16–19, 35). Interestingly, one of these studies demonstrated that patients with acute respiratory distress syndrome (ARDS) due to Covid-19 developed PE significantly more often than patients with ARDS due to other diseases (11.7% vs. 2.1%) (18). Another study showed a much higher frequency of PE in ICU patients with Covid-19 (21%) than during the same time interval in 2019 (6%), and it was also higher than the incidence of PE in patients with influenza admitted to the same ICU in 2019 (8%) (12). It seems clear therefore that the incidence of PE in patients admitted to ICU with Covid-19 is much higher than in other critically ill non-Covid-19 patients, including those with ARDS and other respiratory infections, despite the fact that these patients are already at an increased risk of PE (10).

The only previous study that has addressed the incidence of PE in all patients admitted to hospital with Covid-19 found a percentage of 2.8% (13). Despite the smaller sample size, this proportion is remarkably similar to that found in the present study (2.6%). In both studies, the vast majority of patients (>70%) were admitted to conventional wards. An incidence of PE close to 3% (which may be underestimated by the number of patients undergoing CTPA) is certainly high, particularly when compared with the figure of 1.4% for PE found in a large prospective study of critically ill patients in the ICU (36).

In most of the studies mentioned above, as was the case in the present study, patients developed PE even though most of them were receiving anticoagulant thromboprophylaxis. These findings raise the need to increase thromboprophylaxis doses, particularly in higher risk patients (5, 16, 18). The predictive factors for PE found in the present study can help identify patients who may benefit from high prophylactic doses. Patients with C-reactive protein ≥ 150 mg/L and D-dimer ≥ 1,000 could therefore be candidates for increased doses of thromboprophylaxis; if these patients continue to have a persistently elevated or increasing D-dimer, however, CTPA should be considered to diagnose possible PE.

When analyzing all patients with suspicion of PE, we found that increased C-reactive protein levels (an acute phase protein whose serum concentrations increase during inflammatory states and whose expression is driven by IL-6) (37) correlated with increased D-dimer levels, suggesting a link between inflammation and procoagulant changes, as proposed by other authors (5, 38). In fact, increased C-reactive protein (≥150 mg/mL) at admission was the strongest predictor of developing PE in multivariable analysis, although peak D-dimer had greater diagnostic capacity according to the ROC

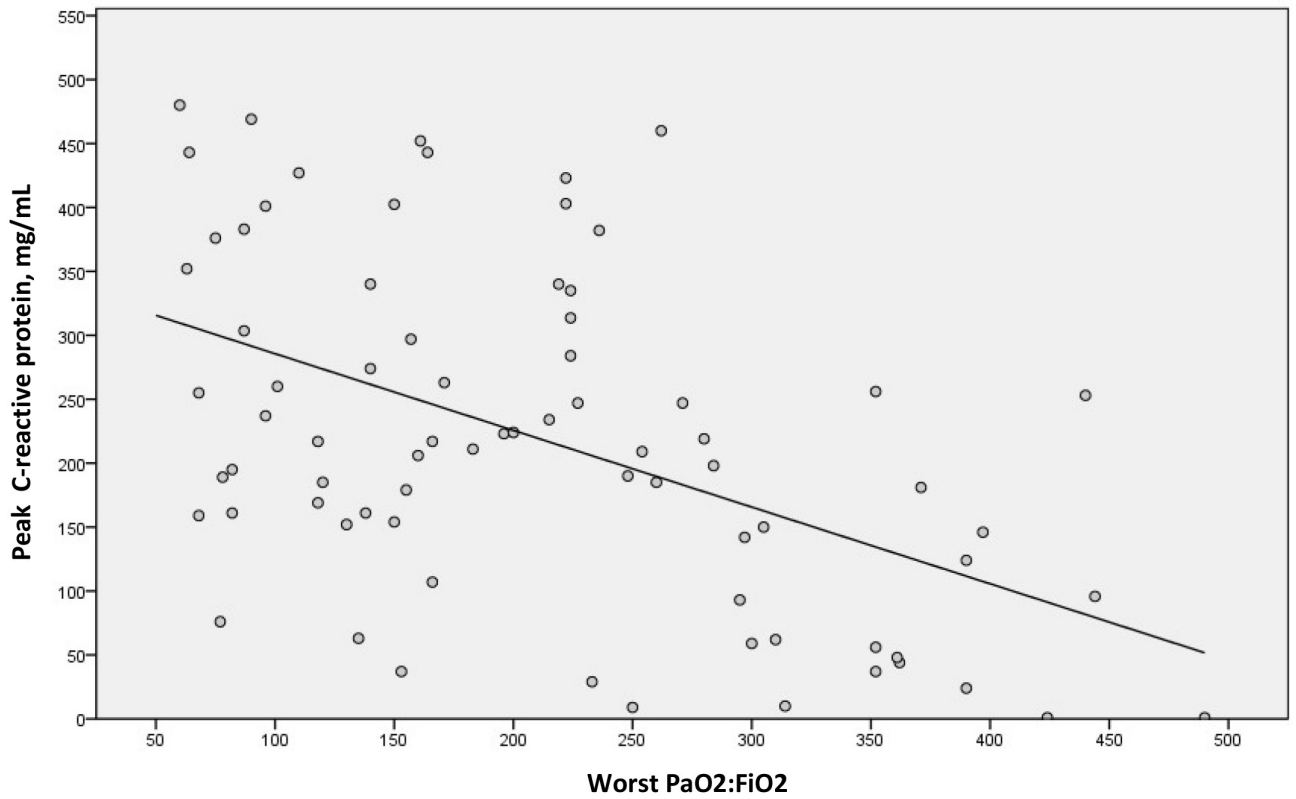


FIGURE 5 | Correlation between highest C-reactive protein and worst PaO_2:FiO_2 during hospital stay in patients with COVID-19 and suspected pulmonary embolism ($r_s = -0.473$; $p < 0.001$).

curve. This supports the hypothesis that inflammation associated with Covid-19 leads to subsequent activation of coagulation and a higher risk of thrombotic disease (4, 38). Furthermore, we also demonstrated that both high levels of D-dimer and C-reactive protein correlated with increased hypoxemia, evidenced by a decrease in the PaO_2:FiO_2 ratio. This provides further important information about the hypercoagulability and thromboinflammatory response associated with Covid-19 and their association with acute lung injury.

A relevant debate has arisen recently about whether thrombosis or pulmonary embolism is the most critical aspect of pulmonary thromboembolic events in Covid-19 patients (16, 39, 40). These patients are clearly at increased risk for venous thromboembolic disease (41), and it is therefore not surprising that they may also develop pulmonary embolisms. Pulmonary thrombosis in Covid-19 patients found at autopsy (42) and, remarkably, in all studied patients in one very recent study (40), however, suggests that pulmonary thrombosis may play a role in the pathogenesis of more severe cases. Our study does not permit to differentiate between thrombosis and pulmonary embolism. Nevertheless, some of our findings would support the predominance of pulmonary thrombosis. First, the low frequency of patients with DVT is worthy of note, which is consistent with other studies (12, 16). Likewise, the predominant involvement in our study of the segmental and subsegmental pulmonary arteries, together with the association between inflammation, coagulation, and hypoxemia, would also support this hypothesis.

TABLE 2 | Characteristics of pulmonary embolism in the computed tomography pulmonary angiogram of patients with Covid-19.

Variable	Patients with pulmonary embolism ($n = 32$)
Location of embolus - no. (%)	
- Main pulmonary artery	0
- Lobar artery	7 (21.9)
- Segmental artery	16 (50)
- Subsegmental artery	9 (28.1)
Thrombus load - no. (%)	
- High	4 (12.5)
- Moderate	8 (25)
- Low	20 (62.5)
Right ventricular overload - no. (%)	5 (15.6)

Our study has limitations that are mainly associated with its observational study design. With such a large number of patients and the high demand for care during this period, systematic performance of CTPA in all patients was not possible, which may have led to underestimating the true incidence of PE. Either way, any large prospective study including all Covid-19 hospitalized

patients should probably be based on establishing criteria by which to evaluate CTPA requests since it does not seem feasible for all patients to have a CTPA. To enable the standardization of criteria, our institution developed hospital guidelines on when to suspect PE and request a CTPA. On the other hand, we compared the features of patients with and without PE on the basis of their CTPA results so that our findings cannot be extrapolated to the entire cohort of inpatients with Covid-19. In order to obtain results that apply to all hospitalized patients with Covid-19, we are currently planning a nested case-cohort study that will include all PE patients diagnosed by CTPA as cases and a random sample of all Covid-19 inpatients as controls. The CT unit used in our study was a Phillips Brilliance CT 16-slice scanner, which is the one reserved at our center for emergencies. During the Covid-19 pandemic, this CT unit was reserved for the study of all patients with Covid-19 in order to spare the other CT units. While better CT image resolution would be obtained with other more technologically up-to-date units, we think that the quality of the image, even in subsegmental arteries, is sufficient for cases of PE. Even so, while the frequency of PE found is high, it is probably underestimated, as the data from autopsies of patients with Covid-19 suggest (40, 43). In an autopsy study of 12 consecutive patients who died from Covid-19, PE was found in five of them even though no preclinical evidence of PE had been reported (43). In another study, meticulous autopsies of 11 deceased patients (10 of whom were selected at random) observed thrombosis in small to mid-sized pulmonary arteries in all cases (40).

In conclusion, patients admitted to hospital with Covid-19 have a high incidence of PE, estimated at 2.6%. Our study identified predictors of PE able to select patients at increased risk of developing PE, making them possible candidates for thromboprophylaxis at higher doses. There is a correlation between increased levels of C-reactive protein and D-dimer and increased hypoxemia, which supports the role of thromboinflammation in acute lung injury observed in patients with Covid-19. We need more information on the most appropriate thromboprophylactic doses and duration to prevent PE in patients with Covid-19.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of the Hospital de la Santa Creu i Sant Pau. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

NB, DF, and JM conceived the study, searched the literature, and designed the study. NB analyzed the data and drafted the report. NB, DF, JM, and AF interpreted the results. DF, VP, JG-A, and AG collected the data and critically revised the report for important intellectual content. DF, JM, and AF critically revised the report. JC and PD supervised the study, interpreted the results, and critically revised the report for important intellectual content. All authors gave final approval of the version to be published.

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BRIEF RESEARCH REPORT
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From Standard to Escalated Anticoagulant Prophylaxis in Fractured Older Adults With SARS-CoV-2 Undergoing Accelerated Orthopedic Surgery

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Proximal femoral fractures in older adults are not uncommon and represent a great challenge for orthopedic surgeons because of the high risks of complications. In the COVID-19 panorama, fractures occurring in infected older adults become an even more intricate task because of concomitant metabolic derangements due to SARS-CoV-2. Multidisciplinary protocols are mandatory and pharmacological treatment in infected patients should be tailored. Regrettably, the spread of the virus in northern Italy, has been faster than scientific progress in characterizing the disease and many hospitals have had to manage the symptoms on a daily clinical bases. Our Italian hospital in the region of Lombardy, which has been the epicenter of the Italian pandemic, has admitted sixteen patients with fractured femurs in March and April 2020. The first seven patients were treated with the antithrombotic prophylaxis of a single daily dose of low-molecular-weight heparin, but we observed the highest prevalence of deaths from cardiovascular complications (four deaths). By doubling the daily dose of anticoagulants in the subsequent patients, we observed a reduction in the incidence of death (one death out of nine). Controversies exist about the surgical treatment of fractures in older adults during this pandemic. However, we have observed an increased survival after fall trauma in infected older adults if treated with high doses of anticoagulant. Although not being statistically significant, our results are in line with the current knowledge of the pathophysiology of SARS-CoV-2 infection, but more studies should be shared about the efficacy and dosage of anticoagulants in traumatic injuries of the elderly.

Keywords: femoral fractures, anticoagulants, low-molecular-weight heparin, COVID-19 drug treatment, SARS-CoV-2

COVID-19 PANDEMIC: TO OPERATE, OR NOT TO OPERATE FRACTURES IN INFECTED OLDER ADULTS?

Italy has been the worst-hit country in Europe during the pandemic of SARS-CoV-2 with 13.6% of mortality rates (WHO situation report 100 of April 29 2020). This disease named COVID-19 represents a severe health-care emergency, leading to a challenging management of trauma patients infected with the virus (1, 2). Italian hospitals have been involved in managing patients suffering from this infection and other comorbid conditions, with the health care system being rapidly saturated. At our orthopedic hospital in the region of Lombardy, which has been the epicenter of the Italian pandemic counting about 50% of all death in the country, has been reorganized to guarantee the best assistance to trauma emergencies (3). During this worldwide plague, the choice of operating older adults who have suffered a falling trauma has been a matter of debate. In the worst-hit countries, the experience of Chinese (4), Spanish (5), and colleagues from England (6) would suggest delaying the surgical treatment of fractured patients with SARS-CoV-2 or treating conservatively, as they have observed excessive mortality rates. The Italian experience would conversely suggest treating the fracture as soon as possible in order to stabilize the patient (7). Nevertheless, the choice depends on the type and severity of the fracture and is a fact that most of older adults that encounter a falling fracture face a life-treating event that need to be treated. For these patients, surgery has to be ensured within 48 h from admission (8) and supported with prophylactic anticoagulant therapy for venous thromboembolism consisting of a daily administration of low-molecular-weight heparin (LMWH, 4000 IU), which has been shown to decrease the risk of events by over 30% (9). The aim of this article is to share our anticoagulant management of fractured older adults infected with SARS-CoV-2 in order to discuss new criteria for the correct prevention of thrombotic events in COVID-19 patients. The ethical review and approval was not required for this case series study in accordance with the local legislation and institutional requirements.

METHODS IN THE TIME OF COVID-19

In March and April 2020, our orthopedic hospital counted sixteen emergency operations of non-deferrable femoral neck fractures. At admission, patients were tested for SARS-CoV-2 and categorized according to the 4-level classification of infected patients (therefore positive for the swab): level 0, asymptomatic, the patient should not be hospitalized; level 1, mild symptoms, pharyngodynia, dry cough, fever; level 2, moderate symptoms, high fever, persistent dry cough, asthenia, dyspnoea, requires oxygen support (non-invasive); level 3, severe symptoms, oxygen therapy (invasive), requires access to intensive care (10). Other than the collection of demographic data, clinical evaluations, and biochemical parameters, three classification indices were calculated comprising the CCI (Charlson Comorbidity Index) (11), the MUST (Malnutrition Universal Screening Tool) (12), and the ASA (American Society of Anesthesiologists). Patients received hydration, hydroxychloroquine treatment, and the anticoagulant therapy with LMWH. After surgery, all patients followed the early (within 48 h) physical exercises with physiotherapists since their functionality seemed not excessively altered by the concomitant infection.

RESULTS: FROM STANDARD-DOSE TO ESCALATED-DOSE OF ANTICOAGULANT

At admission, the implemented access plan of the hospital assured an appropriate division of infected, probably infected, and non-infected subjects. At admission, the whole cohort counted two males and fourteen females with a mean age of 86.4 ± 6.2 years old all suffering of at least one cardiovascular condition (CCI $= 4.4 \pm 0.7$), such as ischemic heart disease, hypertension, atrial fibrillation, or a combination of three. All patients resulted positive for SARS-CoV-2 and most of the subjects were disoriented in time and space rendering the proper assessment of COVID-19 symptoms hard to evaluate. The mild impairment of the peripheral oxygen saturation (all had over 90%) and the infective status allowed us to categorize the subjects as level 2 COVID-19 severity. All patients moderately suffered from limited activity (ASA score $= 2.9 \pm 0.4$) and malnutrition (all were at high risk of malnutrition). Concerning the therapy with LMWH, the first seven patients were routinely supported with a standard prophylactic dose of 4000 IU. In particular, five patients received daily administration of Enoxaparin sodium and two patients received an equivalent prophylactic dosage of Nadroparin calcium based on the body weight. However, their clinical situation deteriorated rapidly within a few days and four patients had fatal cardiovascular events, being one ischemic stroke, two cardiac arrests in heart failure, and one pulmonary embolism. The observed cardiovascular mortality possibly associated with thrombotic events and the concomitant proposal of some scientific publications that suggested the prevalent hemostatic involvement in response to the virus were fundamental in deciding to double the dose of anticoagulant. In agreement with the hospital's health management, it has gone from standard-dose to escalated-dose of antithrombotic therapy. Therefore, the subsequent patients admitted for femoral neck fractures (nine consecutive patients) were treated with 4000 IU of Enoxaparin sodium twice a day. Still, one patient died for ischemic stroke. Overall, this second series was comparable to the first series for age (equal variances T-test: $p = 0.107$), malnutrition risk, comorbidity index, anesthesiology risk, and level of COVID-19 severity. No differences in the biochemical parameters at admission was found: hematocrit (equal variances T-test: $p = 0.855$), hemoglobin (equal variances T-test: $p = 0.904$), platelet count (equal variances T-test: $p = 0.142$), white blood-cell count (equal variances T-test: $p = 0.732$), C-reactive protein (equal variances T-test: $p = 0.258$), urea (equal variances T-test: $p = 0.708$), creatinine (equal variances T-test: $p = 0.950$), AST (equal variances T-test: $p = 0.324$), ALT (equal variances T-test: $p = 0.180$) (see **Table 1** and **Figure 1** for details). Despite not being statistically significant (2-sided Fisher's exact test: $p = 0.106$), the death rate decreased from 57.1 to 11.1%, which is

TABLE 1 | Data of older adults admitted to an Italian orthopedic hospital for femoral neck fracture in April 2020.

	Standard anticoagulant therapy, single daily dose of LMWH	**Escalated anticoagulant therapy, double daily dose of LMWH**
General demographics		
Femoral fractured patients	Raw of 7	Raw of 9
Age (years)	89.3 ± 3.9 (82.0, 95.0)	84.2 ± 6.9 (72.0, 93.0)
Gender (ratio male:female)	Ratio 0:7	Ratio 2:7
Surgery period	First half of April 2020	Second half of April 2020
Parameters at admission		
SARS-CoV-2 quantitative RT-PCR	100% positive	100% positive
Peripheral oxygen saturation	100% over 90%	100% over 90%
Malnutrition Universal Screening Tool (MUST)	100% high risk	100% high risk
American Society of Anesthesiologists (ASA)	3.0 ± 0.6	2.9 ± 0.3
Charlson Comorbidity Index (CCI)	4.4 ± 0.8	4.4 ± 0.7
COVID-19 level of severity	100% level 2	100% level 2
Hematocrit (%)	35.6 ± 5.9 (29.8, 44.2)	36.2 ± 5.9 (27.8, 45.0)
Hemoglobin (g/dL)	11.9 ± 2.2 (9.6, 15.5)	11.7 ± 1.9 (8.9, 14.3)
Platelet count ($10^3/\mu L$)	218.9 ± 85.2 (69.0, 316.0)	293.2 ± 101.4 (174.0, 469.0)
White blood-cell count ($10^3/\mu L$)	10.4 ± 4.0 (4.3, 15.6)	11.2 ± 5.5 (4.0, 23.1)
C-reactive protein	2.5 ± 3.6 (0.1, 8.7)	4.6 ± 3.7 (0.0, 10.3)
Urea (mg/dL)	48.6 ± 20.2 (28.0, 83.0)	53.8 ± 31.1 (19.0, 120.0)
Creatinine (mg/dL)	0.8 ± 0.3 (0.5, 1.4)	0.8 ± 0.4 (0.3, 1.5)
AST (U/L)	32.4 ± 15.2 (18.0, 60.0)	25.3 ± 12.6 (14.0, 49.0)
ALT (U/L)	22.3 ± 12.9 (8.0, 45.0)	15.7 ± 5.2 (7.0, 23.0)
Clinical outcome		
Length of hospital stay	10.4 ± 4.9	14.7 ± 8.6
Discharged with recovery	3	8
Deceased	4	1

CCI scores 1–2 = mild. CCI scores 3–4 = moderate. CCI scores ≥ 5 = severe.
COVID-19 level of severity: classification of the infected patient (therefore positive for the swab) according to 4 levels at admission. Level 0: asymptomatic, the patient should not be hospitalized. Level 1: mild symptoms, pharyngodynia, dry cough, fever. Level 2: moderate symptoms, high fever, persistent dry cough, asthenia, dyspnoea, requires oxygen support (non-invasive). Level 3: severe symptoms, oxygen therapy (invasive), requires access to intensive care.

a remarkable outcome different. Since autopsies were currently suspended for biohazard security concerns, all causes of death have been determined based on clinical evaluations. We found no statistical difference between survivors and non-survivors with regard to any routine biochemical parameter, which resulted to be rather normal for the conditions, or clinical symptom at admission. Although also the age between the single- and double-dose LMWH groups was not statistically different, the survivors had a mean of 84.6 ± 6.6 years vs. the 90.4 ± 2.7 years of non-survivors. By the end of April, eleven (68.8%) patients have been discharged and five (31.3%) patients have died.

DISCUSSION

We here compared the survival in two consecutive series of patients, among them overlapping for clinical conditions and hospital path, which have undergone two different perioperative dosages of LMWH for proximal femoral neck fracture in March-April 2020. Notably, all patients resulted to be at high risk of malnutrition, being the alteration of the nutritional status a predisposing factor both for SARS-CoV-2 infection (13) and traumatic falls (14). Despite the slight similarity, the survivors were younger than those deceased and it is known that the older the age the higher the case fatality in individuals suffering from COVID-19 (15). The low platelet count is also known to be associated with increased mortality in patients with COVID-19 (16), but we found no differences between the group of survivors and deceased (260.0 ± 79.3 $10^3/\mu L$ and 262.2 ± 145.4 $10^3/\mu L$, respectively). Of note, the patient that died of cardiac arrest in the first series of patients had thrombocytopenia of 69.0 $10^3/\mu L$ and accounted for the high standard deviation of the group. Our results show that the use of higher doses of LMWH may be associated with improved survival in older adults with COVID-19 after surgical treatment for fracture, but the lack of biochemical monitoring does not allow to conclude that the tendency to reduce mortality is due to a higher dose of anticoagulant. Notably, the number of transfusions in the escalated heparin group was more than double that in the standard dose group (18 vs. 8), definitely reflecting the drug-depended prolongation of the time that blood takes to clot. To the best of our knowledge, this is the first report in Orthopedics discussing the possible advantages

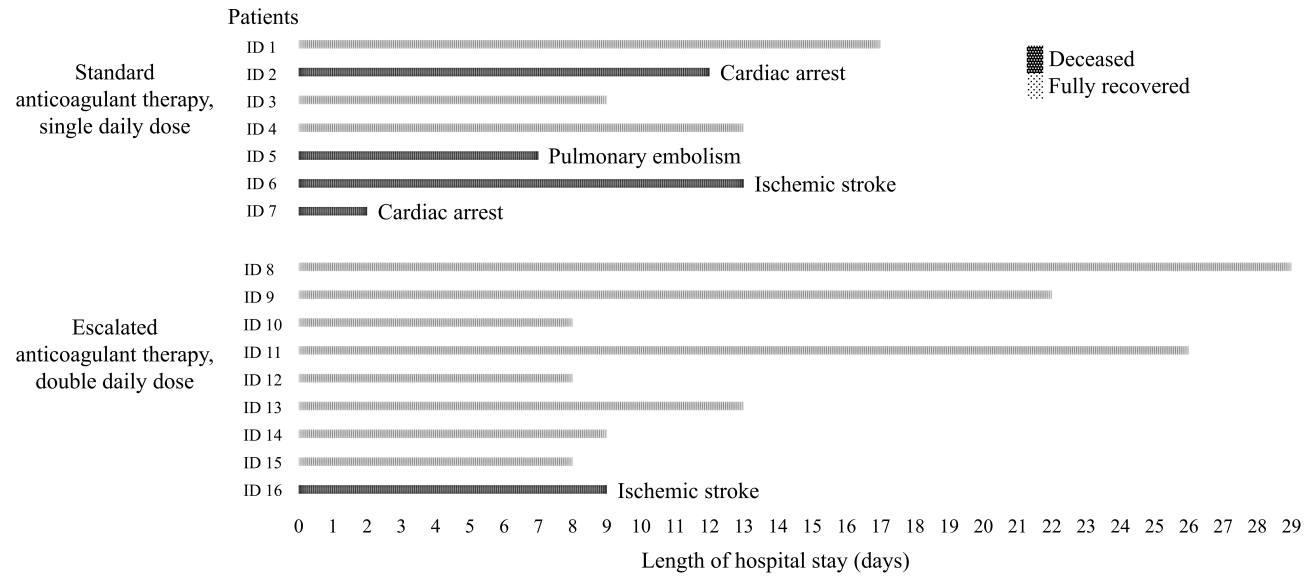

FIGURE 1 | Survival outcome of older adult patients with SARS-CoV-2 after surgical treatment of femoral neck fracture and different anticoagulant therapy. During the months with the highest transmission and death rates for COVID-19 in Italy, sixteen older adults were admitted to an orthopedic hospital of northern Italy for a proximal femoral neck fracture. At admission, all patients resulted positive for SARS-CoV-2, with altered peripheral oxygen saturation, malnourished, mainly suffering from cardiovascular comorbidities, and with a level 2 severity for COVID-19 classification. The first consecutive seven patients were treated according to the standard anticoagulant prophylaxis, but four patients deceased for cardiovascular-associated causes. It was decided to double the dose of low-molecular-weight heparin for the subsequent patients and the death rate decreased from 57.1 to 11.1%.

on survival of higher doses of LMWH compared to standard prophylaxis. Despite our observation cannot be generalized beyond the context of patients' cases, it appears reasonable that an escalated dose of anticoagulant would effectively protect the surgical patient from venous thromboembolism associate with SARS-CoV-2 infection. In this regard, more attention should be paid on the associated bleeding risk. Many severe COVID-19 patients were reported to meet the criteria for the disseminated intravascular coagulation (consumptive of both platelets and clotting factors) (17). In these patients, clot formation was associated with spleen atrophy, hilar lymph node necrosis, and hepatomegaly (18). Whatever is the mechanism underlying the infection-associated thrombosis (that is likely to be multifactorial), the coagulopathy observed in COVID-19 patients is likely to follow the "two-activation theory of the endothelium" (19), with both the release of inflammatory cytokines and the activation of platelets that trigger the activation of inflammatory and microthrombotic pathways. This cascade of events has been observed in severe influenza pneumonia (20), but it is also known to be the underlying pathogenesis for ischemic heart diseases (acute coronary syndrome), stroke, and venous thromboembolism (which includes deep vein thrombosis and pulmonary embolism).

Current guidelines from the National Institutes of Health state that there is still insufficient data to recommend either for or against using escalated or therapeutic doses of antithrombotic agents in infected patients, and still the evidences are based on expert opinions (21–23). The clinical efficacy of heparin in decreasing mortality rates in COVID-19 has been suggested by colleagues from China and United States of America (24–26), but they did not discuss the dose per patient though. It should be considered that most of the evidences on the use of anticoagulants has not been based on trauma older adults. Notably, these patients normally face an already very high risk of basal thromboembolic complications and we therefore believe that surgery with a double dose of anti-thromboembolic prophylaxis represents the most appropriate treatment for these patients with femoral fractures. Although our results should not be used to support changes in clinical practice because of the observational nature of the study and the lack of statistical significance, older adults receiving a standard dose of anticoagulant were more at risk of death than those receiving a double dose (relative risk 5.14, CI 95% 0.73:36.37). In the near future, we are looking forward to seeing results from large prospective randomized trials that investigate whether higher doses of LMWH would protect against thrombotic events in COVID-19 patients undergoing surgery. Monitoring of coagulation parameters and bleeding events should be included. In addition, it would be interesting to focus research on the potential antiviral role of heparin (27).

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories

and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

PP formulated the hypothesis. RG and MB wrote the first draft of the manuscript. MZ, RA, LM, GP, and PP revised the first draft and contributed to manuscript sections. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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CASE REPORT
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Locked-in Syndrome in a Young Patient Due to SARS-CoV-2: A Case Report

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Coronavirus disease 2019 (COVID-19), apart from commonly involving the respiratory system, has its impact on the central nervous system, with a wide spectrum of clinical presentations ranging from headaches to ischemic strokes. The ongoing research regarding this novel disease has found that there is a very high prevalence of thrombotic episodes especially in critically ill patients when compared to severe presentation of other viral illnesses. This COVID-19-associated coagulopathy has a very complex etiology with the ability to form thrombus in arteries, veins, and microvasculatures of different organs. We present a unique case of a young woman with underlying COVID-19 who unfortunately developed locked-in syndrome due to bilateral pontine infarction during the course of her illness.

Keywords: COVID-19, SARS- CoV-2, thrombotic complication, hypercoagulability, locked-in syndrome

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INTRODUCTION

Locked-in syndrome is a state of motor paralysis involving all the voluntary muscles of four limbs along with dysarthria, however with preserved alertness and consciousness. This tragic state is rare and often caused by ischemic stroke in midbrain affecting cortico-spinal, cotico-bulbar, and cortico-pontine neuronal tracks. This is the very first case report of its kind in which the cause of ischemic stroke was this novel viral disease. Severe acute respiratory syndrome coronavirus-2019 (SARS-CoV-2019) infection that emerged in Wuhan, China in December 2019 (1) has been declared a global pandemic by World Health Organization (WHO). Where most patients with COVID-19 present with symptoms of cough, fever, fatigue, and dyspnea (2), some also have neurological manifestations like headache, anosmia, meningitis, encephalitis, Guillain–Barré syndrome, and acute cerebrovascular diseases (3). There have been many cases of ischemic stroke that have been reported in patients with confirmed COVID-19. There have been young patients without any underlying risk factors studied that developed ischemic stroke, indicating that COVID-19 has a major role in causing these ischemic strokes (4–7). Mao et al. reports that acute cerebrovascular accidents are more common in patients having a severe COVID-19 disease compared to those with less severe disease (8). Our patient uncharacteristically developed locked-in syndrome despite initially presenting with respiratory symptoms of worsening dyspnea and relentless productive cough.

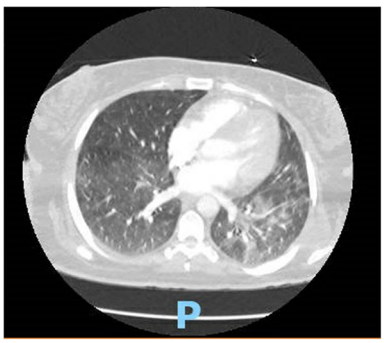

FIGURE 1 | CT chest showing typical BL ground glass opacifications in lungs: a hallmark of SARS-CoV-2 infection.

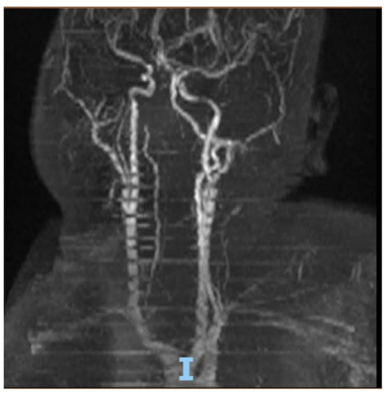

FIGURE 2 | MRA neck showing patent circulations of carotid arteries.

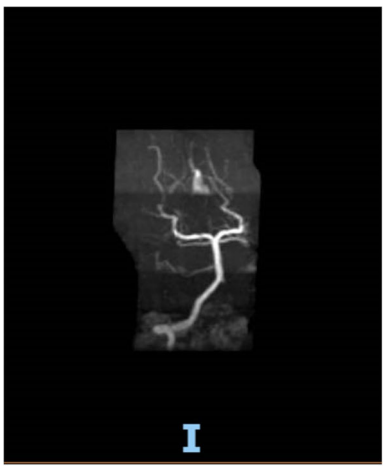

FIGURE 3 | MRA brain with occluded right vertebral artery and patent basilar artery.

CASE

A 25-year-old woman with past medical history of hypertension and diabetes mellitus type I presented to the Emergency Department (ER) with symptoms of dry cough, low-grade fever, and worsening shortness of breath for 1 week. In the emergency room, the triage vital signs showed that she was hypoxic to 70% on pulse oximetry, which improved to 96% on 6 L of supplemental oxygen via nasal cannula. Considering the COVID-19 pandemic and her typical symptoms, a nasopharyngeal swab for COVID-19 PCR was done in the ER, which eventually came out as positive.

On hospital day 2, she developed acute respiratory syndrome (ARDS), and she eventually had to be intubated requiring mechanical ventilation. Computed tomography of the chest showed interstitial infiltrate dictating the severity of the patient (**Figure 1**). Eventually, after 8 days of requiring high fraction of oxygen (FiO_2) up to 100% and positive end expiratory pressure (PEEP) of >12, her lung compliance started to improve, and we were able to decrease her FiO_2 and PEEP requirements. She was off sedation, and we attempted several unsuccessful spontaneous awakening and breathing trials. To evaluate her for unresponsiveness despite being off sedation, a computed tomography of the head without contrast and electroencephalography were done, which came back as unremarkable for any acute findings.

On hospital day 16, the patient finally opened her eyes and started following simple commands such as blinking of her eyelids. She was just able to respond to any command by her vertical eye ball movement and blinking of eyelids but continued to not show any movement in all four of her extremities. A repeat CT scan of the head on day 16 was also unremarkable for any acute intracranial pathology. Neurology was consulted; after 6 days off sedation on physical examination, patient was arousable to voice and tactile stimulation by opening of her eyes and was able to track objects with eye. Bilateral pupillary reflex, corneal reflex, doll's eye reflex, and gag reflex were intact. The patient's National Institutes of Health Stroke Scale (NIHSS) score was 27, showing severe stroke with total motor impairment of all extremities. A magnetic resonance imaging of the brain to rule out stroke, a magnetic resonance angiography to rule out arterial stenosis, an echocardiography to rule out cardiac source of any emboli, and a lumbar puncture for cerebrospinal fluid analysis were done. The results of the magnetic resonance angiography (MRA) of the neck (**Figure 2**), echocardiography, and cerebrospinal fluid (CSF) analysis was unremarkable; however, MRA of the head (**Figure 3**) and a non-contrast MRI of the brain (**Figure 4**) showed multiple foci of restricted diffusion within the pons, compatible with acute infarcts.

Patient was on deep vein thrombosis (DVT) prophylaxis Heparin 5,000 U subcutaneous every 8 h from day 1 of hospitalization. After finding acute infarct and occluded vertebral artery, full anticoagulation with intravenous Heparin 16 U/h was started with the goal of activated partial thromboplastin time (aPTT) of 60–90 s. As shown (**Figure 3**), there was a decreased flow in the distal right vertebral artery suspicious of blood vessel occlusion. Basilar artery is patent on the MRA (**Figure 3**). Given the bilateral nature of acute pontine central infarct as shown in MRI (**Figure 3**), the culprit basilar thrombus originated from the occluded right vertebral artery, was stuck for a time

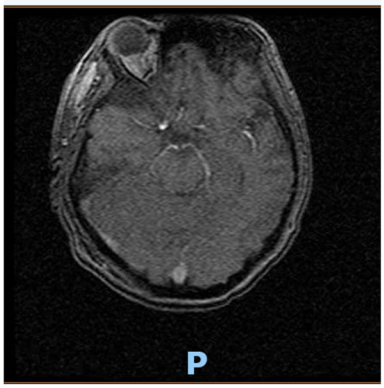

FIGURE 4 | MRI brain (cross-section at pons) consistent with acute infarct of pons.

being in the basilar artery, lead to bilateral stroke, and resolved later (**Figure 3**). It is very less likely that a bilateral pontine infarction, leading to locked-in syndrome, can be caused by direct thromboembolic occlusion of basilar perforator arteries.

Neurologist recommended full hypercoagulable workup including platelets, protein S, protein C, antithrombin-III, PT, aPTT, d-dimer, antiphospholipid (APL) antibody isotypes, fibrinogen, and fibrin split products. Normal platelet count of around 285,000/μl and normal PT and aPTT excluded disseminated intravascular coagulation (DIC). Raised inflammatory markers, i.e., fibrinogen level, d-dimers, CRP, and ferritin are the hallmark of cytokine storm leading to sepsis-induced coagulopathy. Considering the area of ischemic stroke and the fact that there was no improvement from locked-in syndrome after 29 days, on the 30th day, a tracheostomy and percutaneous endoscopic gastrostomy was performed, and the patient was sent to a nursing home later on minimal ventilator settings.

DISCUSSION

We describe a case of locked-in syndrome secondary to COVID-19, leading to a tragic state of quadriplegia with sparing of consciousness and eye movements with clinical, serological, and neuroimaging evidence. The infarction of the midbrain at central pons can lead to this quadriplegic state known as locked-in syndrome (9), and this could be the very first case reported where COVID-19-associated coagulopathy led to a pseudocoma state of locked-in syndrome with acute, isolated bilateral pontine infarction. To the best of our knowledge, only one case of COVID-19-associated locked-in syndrome has been reported and that was due to acute polyradiculoneuropathy (10). The systemic infection of SARS-CoV-2 can lead to cytokine production (mainly IL-6) called "cytokine storm" as a part of innate immunity (11). The cytokine inflammatory response leads to activation of procoagulation pathways. Phosphatases (derived from viruses) activate platelets, mast cells, and factor XII (FXII), causing hypercoagulation through activation of intrinsic coagulation pathway. This also results in elevation of D-dimers, fibrinogen level, and C-reactive protein (12). The cytokines also activate endothelial cells with resultant endothelial injury, leading to microthrombi formation in vessels with subsequent ischemia and multiple organ failure (13). Another pathophysiological mechanism through which SARS-CoV-2 can cause acute cerebrovascular accidents is through endothelial injury mediated by depletion of angiotensin-converting enzyme 2 (ACE2). ACE2 receptors are expressed on lungs, intestine, and brain (14). Overexpression of ACE2 in neuronal cells or endothelial progenitor cells protects the brain from ischemic stroke (15). ACE 2 is cardio- and neuroprotective and acts by countering the effects of angiotensin converting enzyme 1 (ACE1) and angiotensin 2(AT2) in the renin–angiotensin–aldosterone system. SARS-CoV-2 binds to ACE2 receptors and depletes ACE2, leaving ACE1 unopposed with production of AT2, which worsens lung injury with proinflammatory and organ damaging effects.

The possible mechanism of COVID-19-associated coagulopathy and thrombus formation therefore can be summarized by four major mechanisms. The first is COVID-19-induced cytokine storm that activates host immune defense in order to protect the spread of the virus, leading to activation of coagulation cascade in the blood. The second is platelet activation by these proinflammatory cytokines. Third would be direct endothelial involvement causing apoptosis of endothelial cells exposing the subendothelial matrix, which acts as a potent trigger for platelet aggregation and thrombus formation. The last one could be because of fibrinolytic suppression caused by decreased activity of plasminogen activator and elevated release of plasminogen activator inhibitors. All these mechanisms are hence responsible for "sepsis induced coagulopathy," which is related with the severity of COVID-19 (16, 17).

CONCLUSION

Physicians including neurologists should be aware of the fact that COVID-19 disease can cause acute ischemic strokes. The control of COVID-19 is our biggest priority currently, but we should not neglect COVID-19-associated strokes because early provision of anticoagulation can decrease the long-term mortality and morbidity in these patients. It has now been advised that prophylactic use of anticoagulants should be given to ameliorate the potential risk of hypercoagulopathy associated with COVID-19. Some physicians are also advising to add antiplatelet drugs to prevent arterial thrombus formation, but the risk of bleeding could be a limiting factor in this practice. Further research is warranted to understand the COVID-19-associated coagulopathy and thrombus formation in arteries to prevent fatal episodes of acute coronary and cerebrovascular accidents like the one seen in our patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

A written, informed consent was obtained from the patient/legal representative for the publication of this case report (including all data and images).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Coagulopathy as a Prodrome of Cytokine Storm in COVID-19-Infected Patients

Hui Guo[1†], Ying Sheng[2†], Wei Li[3], Fei Li[4], Zongyu Xie[5], Jing Li[1], Yuhe Zhu[6], Jian Geng[6], Gang Liu[7], LeJian Wang[8], Jing Li[9] and Fengchao Wang[1*]*

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Background: The rapid coronavirus disease 2019 (COVID-19) pandemic has hit hard on the world and causes panic since the virus causes serious infectious respiratory illness and easily leads to severe conditions such as immune system overactivation or cytokine storm. Due to the limited knowledge on the course of infection of this coronavirus and the lack of an effective treatment for this fatal disease, mortality remains high. The emergence of a cytokine storm in patients with a severe condition has been reported as the top reason of the death of patients with COVID-19 infection. However, the causative mechanism of cytokine storm remains elusive. Thus, we aim to observe the association of coagulopathy (D-dimer) with cytokine (i.e., IL-6) and CT imaging in COVID-19-infected patients.

Methods: In this retrospective observational study, we systematically analyzed the comprehensive clinical laboratory data of COVID-19-positive patients in different illness groups of mild, moderate, and severe conditions according to the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition). *T* tests and chi-square tests were used for two-group comparisons. One-way ANOVA was used for three-group comparisons. Pearson and Spearman correlation coefficients of the D-dimer level with IL-6 and CT imaging were computed at baseline. With regular liquid biopsy approach, D-dimer, IL-6, and neutrophil-to-lymphocyte ratio were recorded repeatedly with a time curve to investigate disease progression, along with CT imaging, and other indicators.

Results: All the 64 patients were clinically evaluated and classified into three groups of mild (32 cases), moderate (23 cases), and severe (nine cases) conditions. The D-dimer level positively correlated with IL-6 ($R = 0.5$) at baseline when the COVID-19-infected patients were admitted. In addition, we observed that D-dimer rises earlier than the cytokine storm represented by IL-6 surge, which suggests that coagulopathy might act as a trigger to potentiate a cytokine storm.

Conclusion: Integrated analysis revealed a positive correlation of coagulopathy with cytokine storm in COVID-19-infected patients; the D-dimer rises early, which indicates that coagulopathy acts as a prodrome of cytokine storm. Coagulopathy can be used to monitor early cytokine storm in COVID-19-infected patients.

Keywords: COVID-19, coagulopathy, cytokine storm, prodrome, d-dimer, IL-6

INTRODUCTION

COVID-19 has become a global pandemic. The coronavirus is a large virus family, which was known to cause serious infectious respiratory illnesses such as Middle East respiratory syndrome, severe acute respiratory syndrome (SARS), and SARS-CoV-2 (1–5). SARS-CoV-2 infects humans via the same receptor as SARS-CoV—human angiotensin converting enzyme II (6). Coronavirus disease 2019 (COVID-19) is a disease widely spread across continents and oceans, which causes severe damages to the human body and panic in the world. Due to the limited knowledge on the course of infection of this coronavirus, the mortality of COVID-19-infected patients remains high (7, 8). Cytokine storm was observed and is considered as the top reason of death in COVID-19-infected patients (9–11). The unexpected emergence of a cytokine surge frequently appears in patients with severe conditions of pneumonia (11–13). However, the causative origin of a cytokine storm is unknown. The elusive trigger of a cytokine storm renders effective prevention and treatment impossible. Therefore, we used COVID-19 infected patients as a model to study the origin of a cytokine storm. Identifying the origin of a cytokine storm will enable us to act early to block or decelerate its lethal progression.

Cytokine storm starts locally in the lung and is abruptly activated in the systemic-level circulation, which results in persistent hypotension, hyper-or hypothermia, leukocytosis or leukopenia, and often thrombocytopenia (14). It was implied that the circulation system could be the key step for the ignition of a cytokine storm, promoting an inflammation from a local one to a severe systemic illness (15).

Coagulopathy in patients with COVID-19 has been reported with a high level of D-dimer (16). D-dimer, a degradation product of cross-linked fibrin indicating thrombosis, is widely used as an indicator of global activation of hemostasis and fibrinolysis. The neutrophil to lymphocyte ratio (N/L ratio) has been used as a clinical liquid biopsy marker for systemic inflammatory status in various disease types for many years (17). A correlation between the serum levels of interleukin-6 (IL-6) and the severity of COVID-19 symptoms has been reported (18, 19). The anti-IL-6 antibody tocilizumab is actively being tested in clinical trials by Xu et al. (19); the anti-IL-6 receptor antibody was also expanded in clinical trials for COVID-19 patients by Regeneron and Sanofi. The aim of this study was to observe the association of coagulopathy (D-dimer) with cytokines [i.e., neutrophil-to-lymphocyte ratio (NLR), IL-6, and C-reactive protein (CRP)] and CT imaging in COVID-19-infected patients.

METHODS

Patient Selection

Patients were recruited from an in-patient unit of the First Affiliated Hospital of Bengbu Medical College. Sixty-four patients with a diagnosis of COVID-19 were included in the study. All the 64 patients were clinically evaluated and classified into three groups of mild, moderate, and severe condition with COVID-19 infection according to the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition). There were 32 patients, including 15 males and 17 females, with a mean age of 54 years (range 33–73) in the mild group, 23 patients including 13 males, and 10 females with a mean age of 57 years (range 21–83) in the moderate group, and nine patients including eight males and one female, with a mean age of 61 years (range 47–81) in the severe group. Of the nine patients in the severe group, five died during a follow-up of the study. The ethical committee of the First Affiliated Hospital of Bengbu Medical College approved this study; the approval number is BYYFY-2020KY03.

The 64 COVID-19-positive patients were confirmed to have a viral load by nucleic acid real-time RT-PCR test (commercial kit specific for 2019-nCoV, DaAn Gene Co., Ltd., Guangzhou, China) conducted at least twice.

Laboratory Data Collection

Complete blood count was tested on a blood analysis platform (XE-5000, Sysmex), with white blood cell count and classification using semi-flow fluorescent staining technology. NLR was calculated from the number of neutrophils and lymphocytes. D-dimer was measured by immune turbidimetry on an automated coagulation system (CS-5100, Sysmex). CRP was measured by immune turbidimetry on an automated biochemistry analysis platform (cobas 8000, Roche). IL-6 was tested with electrical chemical immune analysis technology (cobas e601, Roche). Procalcitonin was measured with a fluorescence immunochromatographic assay (QT-200, Wondfo).

Follow-Up

After admission, the patients were routinely monitored for the laboratory results of routine blood test, CRP, and CT imaging when medical treatment was necessary. Follow-up occurred twice on the first month after discharge and then monthly for an additional 3 months.

Statistics

The cutoff value of NLR was calculated based on the maximum Youden index. *T*-tests or chi-square tests were used to compare

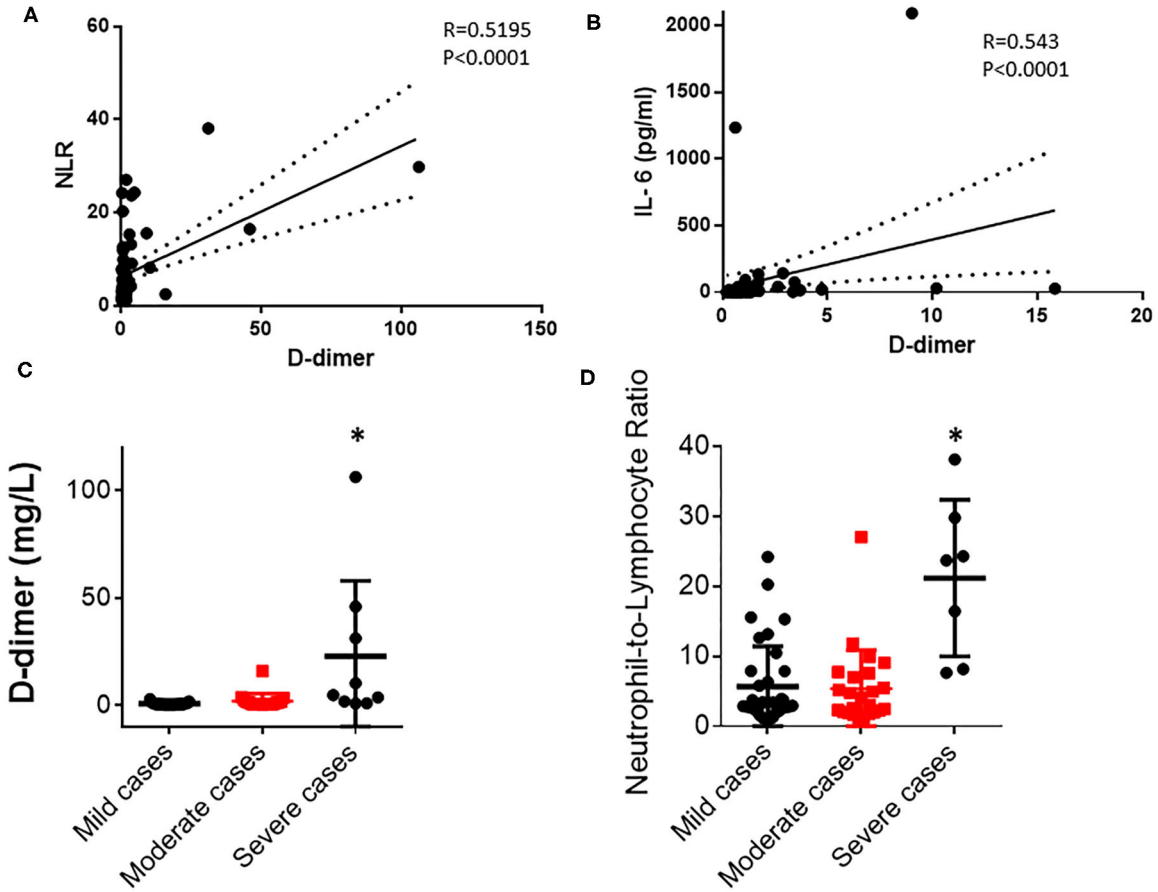

FIGURE 1 | D-dimer correlates with neutrophil-to-lymphocyte ratio (NLR) and IL-6 in COVID-19-infected patients. **(A)** D-dimer level correlates with NLR in all 69 COVID-19 patients (Pearson correlation, $R = 0.5195$, $p < 0.0001$). **(B)** D-dimer level correlates with IL-6 level in the serum of all 69 COVID-19 patients (Spearman correlation, $R = 0.543$, $p < 0.0001$). **(C)** Comparison of D-dimer levels in mild, moderate, and severe groups ($p = 0.0002$). **(D)** Comparison of NLR in mild, moderate, and severe groups ($p < 0.001$). *$p < 0.05$.

differences between two groups. ANOVA tests were used to compare the differences among mild, moderate, and severe groups. Correlations were analyzed with either Pearson or Spearman correlation coefficient. Analyses were performed using SPSS 22.0 statistical package (SPSS, Inc., Chicago, IL, USA) and R version 3.6.2. $P < 0.05$ was considered as statistically significant.

RESULTS

D-dimer Correlates With NLR and IL-6 in COVID-19-Infected Patients

As previously reported, D-dimer levels over 1 μg/L at admission predicted an 18-fold increase in odds of mortality (12). However, the underlying mechanisms are unknown. NLR was widely reported as a biomarker in various types of diseases, including COVID-19 pneumonia. The neutrophil-to-lymphocyte ratio is believed to able to predict the immune status of patients. However, whether it is related with a coagulant system is unknown.

To investigate how D-dimer contributes to the disease progression of a COVID-19 infection, we observed that the D-dimer level exhibited moderate correlations with NLR ($R = 0.5195$, $p < 0.001$; **Figure 1A**; **Table 1**) and IL-6 ($R = 0.543$, $p < 0.0001$; **Figure 1B**, **Table 1**) in COVID-19 infected patients. These data suggest that a coagulant system is highly likely to correlate with the immune status of COVID-19-infected patients. There are no differences in either D-dimer or NLR between the mild and the moderate groups (**Figures 1C,D**; **Table 1**). As expected, D-dimer is significantly higher in the severe group than the mild and the moderate groups (**Figure 1C**; **Table 1**) at baseline. Similarly, NLR is significantly higher in severe cases than in less severe cases (**Figure 1D**). Thus, these data imply a clinical link of coagulopathy with the immune status of COVID-19 patients.

D-dimer Rises Earlier Than IL-6 and NLR Flare/Cytokine Storm During Infection

The abrupt emergence of a cytokine storm in COVID-19-infected patients is believed to be the most dangerous reason of a patient's

TABLE 1 | Baseline characteristics of Laboratory findings of patients with COVID-19.

Characteristic	Normal Range	Mild cases ($n = 32$)	Moderate cases ($n = 23$)	Severe cases ($n = 9$)	*P*-value
M/F		M15/F17	M13/F10	M8/F1	
AGE		54 (33–73)	57 (21–83)	61 (47–81)	0.0736
GLU	3.9–6.1 mmol/L	6.2 (5.71–6.7)	8.45 (7.09–9.82)	9.78 (5.61–13.96)	< 0.0001
LDL	1.07–3.3 mmol/L	2.30 (2.03–2.58)	2.29 (2.01–2.57)	1.52 (1.19–1.85)	0.0094
D-Dimer	0–0.55 mg/L	0.65 (0.42–0.87)	1.91 (0.3–3.51)	22.79 (−4.14–49.72)	0.0002
NLR		4.58 (2.64–6.52)	5.14 (3.6–6.69)	16.05 (6.39–25.72)	< 0.0001
CRP	0–6 mg/L	38.05 (18.5–57.59)	58.72 (33.08–84.37)	147.87 (95.95–199.8)	< 0.0001
PCT	<0.50 ng/mL	0.18 (0.12–0.23)	0.14 (0.12–0.17)	5.99 (−6.6–18.58)	0.0385
IL-6	<7 pg/mL	12.3 (2.20–22.39)	18.87 (8.27–29.39)	55.49 (−28–139)	0.0197
LY[#]	(1.1–3.2) * 10^9/L	1.34 (1.14–1.55)	1.12 (0.86–1.38)	0.64 (0.33–0.96)	0.0056
MCH	27–34 pg	31.43 (30.71–32.15)	30.41 (29.36–31.46)	30.89 (29.21–32.57)	0.2394
MCHC	316–354 g/L	351.25 (345.6–356.9)	350.91 (342.6–359.2)	346.44 (337.5–355.4)	0.3088
MCV	82–100 fL	89.53 (87.91–91.14)	86.69 (84.52–88.86)	89.23 (83.89–94.58)	0.117
MPV	5.0–11.0 fL	9.525 (8.94–10.11)	9.38 (8.76–10)	9.54 (8.2–10.88)	0.9342
NEUT[#]	(1.8–6.3) * 10^9/L	4.96 (3.22–6.69)	4.29 (3.61–4.96)	8.16 (4.8–11.52)	0.0438
PLT	(125–350) * 10^9/L	247.16 (212.9–281.4)	269.13 (225–313.2)	172.11 (105.8–238.4)	0.0439
RDW-CV	11–15%	12.72 (12.5–12.94)	12.81 (12.5–13.12)	13.12 (12.2–14.04)	0.3739
RDW-SD	37–54%	40.93 (40.04–41.83)	39.83 (38.84–40.83)	41.82 (38.78–44.87)	0.1277
RET[#]	(0.024–0.084) * 10^{12}/L	0.04 (0.029–0.045)	0.034 (0.027–0.04)	0.028 (0.019–0.036)	0.4071
WBC	(3.5–9.5) * 10^9/L	6.92 (5.18–8.67)	5.89 (5.08–6.7)	9.17 (5.64–12.69)	0.1213
A/G	1.2–2.4	1.5 (1.36–1.64)	1.44 (1.24–1.64)	1.27 (1.01–1.52)	0.3197
AG	8–16 mmol/L	13.06 (11.71–14.4)	13.2 (11.41–14.98)	15.32 (11.07–19.57)	0.3285
ALB	40–55 g/L	39.13 (37.85–40.42)	37.12 (35.5–38.74)	33.54 (30.09–37)	0.0008
ALP	45–125 U/L	44.75 (38.69–50.81)	45.13 (39.07–51.19)	64.56 (44.17–84.94)	0.0109
ALT	9–60 U/L	26.25 (17.66–34.84)	35.65 (16.68–54.62)	281 (−320.9–882.9)	0.0574
APOA	0.9–1.6 g/L	0.93 (0.86–1.01)	0.76 (0.7–0.82)	0.61 (0.56–0.66)	< 0.0001
APOB	0.6–1.1 g/L	0.78 (0.7–0.86)	0.76 (0.67–0.86)	0.55 (0.43–0.66)	0.017
AST	15–45 U/L	27.19 (22.01–32.36)	42.57 (22.88–62.25)	753.1 (−898.3–2405)	0.0434
CA^{2+}	2.11–2.52 mmol/L	2.14 (2.1–2.18)	2.13 (2.06–2.2)	1.94 (1.86–2.02)	0.0005
LDH	125–250 U/L	286.97 (237.5–336.5)	322.96 (239.7–406.2)	1,040.56 (84.01–1997)	0.0004
sdLDL	0.27–1.44 mmol/L	0.80 (0.69–0.90)	0.75 (0.64–0.85)	0.39 (0.27–0.51)	0.0004

[#]*Absolute number.*

death. However, the reason for an unexpected cytokine storm is unknown. The tissue and blood vessel damage could be a trigger of an abrupt cytokine storm. To test this hypothesis, we examined the timing and the temporal course of D dimer, NLR, and IL-6 level evolution in peripheral blood during COVID-19 infection in an individual patient with a severe condition. Surprisingly, D-dimer level surged at day 2 after admission (**Figure 2A**), whereas NLR (**Figure 2B**) and IL-6 (**Figure 2C**) levels started to surge at day 7 after admission in one patient with a severe illness condition. These findings demonstrate that the D-dimer level rises earlier than IL-6 glare/cytokine storm along with COVID-19 disease progression (**Figures 2A–C**). To look for more evidences of this temporal course of D-dimer- and IL-6-represented immune activations, we found that D-dimer rose at day 2 (**Figure 2D**) and NLR and IL-6 surged at around day 5 in another patient with a severe condition (**Figures 2E,F**), which suggest that D-dimer-associated tissue coagulopathy might predispose IL-6 production, NLR, and rapid immune overactivation along with COVID-19 disease progression (**Figure 2G**).

D-dimer Correlates With CT Imaging

CT imaging of the lung can reveal the severity of COVID-19 clinical symptoms and is one of the alternative clinical criteria to diagnose a COVID-19 infection. In our cohort of clinical data, the D-dimer level correlated with the ground-glass area of CT imaging and the severity of COVID-19 clinical symptoms at baseline when we compared the D-dimer level with CT imaging in the mild group (**Figure 3A**), the moderate group (**Figure 3B**), and the severe group (**Figure 3C**). The D-dimer level correlated with an increased NLR level from mild to moderate to severe patients. The D-dimer level also correlated with the ground-glass area patterns of CT imaging in patients with mild, moderate, and severe condition, respectively (**Figures 3A–C**).

DISCUSSION

To our knowledge, we are the first to observe that coagulopathy might act as the prodrome of a cytokine storm in COVID-19-infected patients. Coagulopathy appeared around a few days

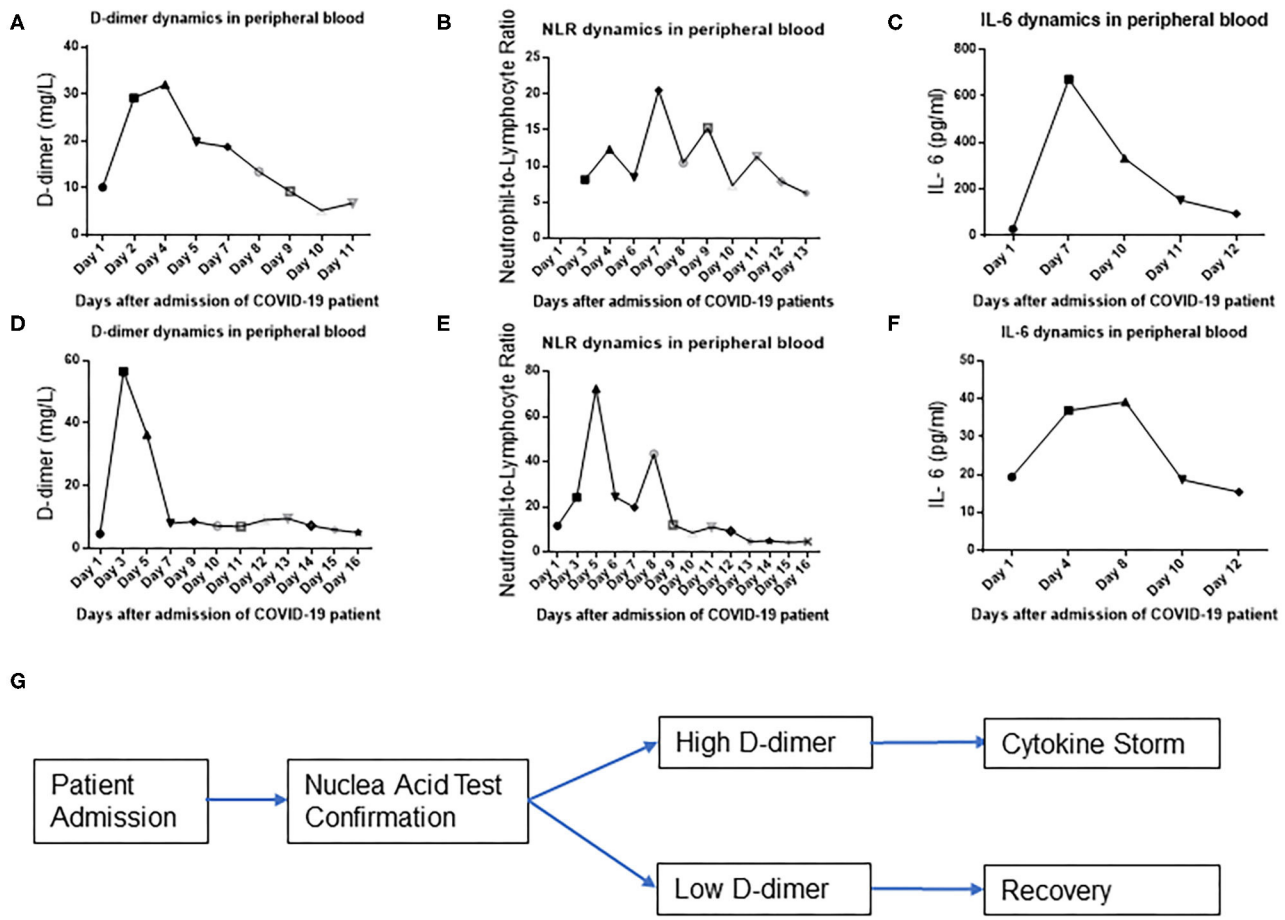

FIGURE 2 | D-dimer precedes IL-6 surge in a severe group of COVID-19-infected patients. D-dimer **(A)**, neutrophil-to-lymphocyte ratio (NLR) **(B)**, and IL-6 **(C)** temporal dynamics of representative patient 1 in peripheral blood. D-dimer **(D)**, NLR **(E)**, and IL-6 **(F)** temporal dynamics of representative patient 2 in peripheral blood. **(G)** Schematic flow diagram of the results.

in advance of a cytokine storm. We also observed moderate correlations of D-dimer with NLR, IL-6 levels, and CT imaging of the lungs in COVID-19-infected patients. D-dimer was reported to correlate with proinflammatory cytokine levels and outcomes in critically ill patients (20). Our data, combined with the results of a previous study (20), might further advance our knowledge of the correlation of coagulopathy and cytokines in human diseases. The D-dimer surge, which is more sensitive than measuring cytokines, might be used to predict a cytokine storm in COVID-19-infected patients. This study indicated the critical clinical value of coagulopathy monitoring and the early requirement of an anti-coagulant therapy to prevent a cytokine storm in COVID-19-infected patients.

A cytokine storm has been observed and considered as the reason of death for COVID-19-infected patients (4, 5). A cytokine storm starts locally in the lungs and gets activated in the systemic circulation; patients must reverse this immune system overactivation (6). It implied that the circulation system is the key step for the ignition of a cytokine storm and the spread of inflammation from local to systemic (7). We reported the blood-system-derived cytokine storm in COVID-19 patients. Considering why the blood system is potent to activate a cytokine storm, if we link it with the basics of immunology, the principle could be antigen dependence—the sudden release of a tremendous amount of antigen provides the power to expand the inflammation into the whole body, systemically activating the immune system and releasing cytokines.

There might be a potential relationship between coagulopathy and neoantigen supply. The systemic immune illness of a cytokine surge requires a rapid mobilization of the human immune system. The toolbox for efficient immune system mobilization in COVID-19 patients remains a mystery. We speculate that coagulopathy might efficiently generate a lot of neoantigens in patients, which helps to efficiently mobilize the human body to over-produce cytokines.

The limitations of our study should be acknowledged. A small sample size from one hospital and the results from this population may not generalize to other populations. A large

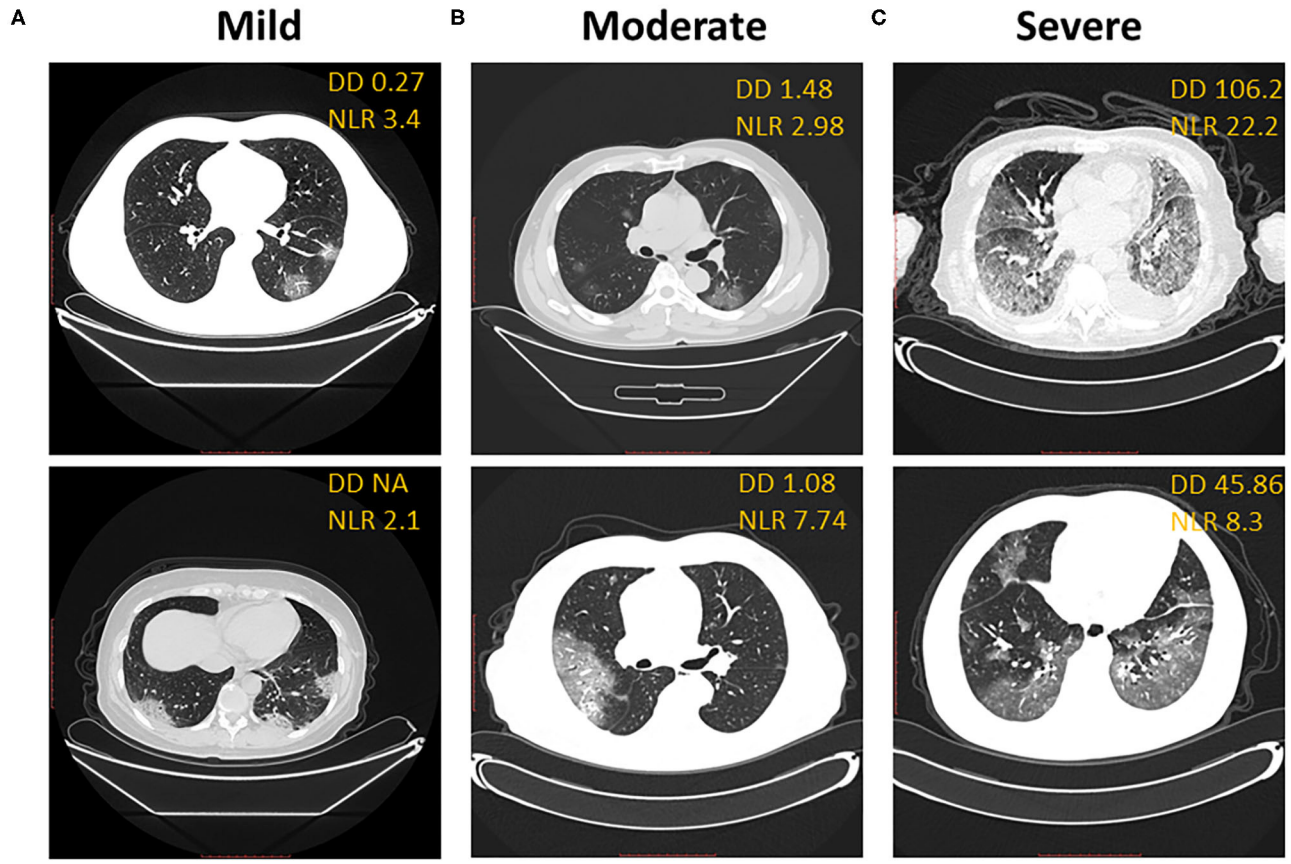

FIGURE 3 | D-dimer correlates with CT imaging. Correlation of representative CT imaging with D-dimer and neutrophil-to-lymphocyte ratio in mild **(A)**, moderate **(B)**, and severe **(C)** groups of COVID-19 patients. **(A)** Ground-glass opacity in the bottom segment of the left lung, blood vessel-like. **(B)** Ground glass shadow expansion and consolidation in bilateral lung. **(C)** Overwhelming ground glass shadow distribution, bilateral patchy shadowing, and enlarged blood vessel.

sample size and more diverse samples are needed to confirm the results. Future studies should analyze more COVID-19 patients from multiple clinical sites and confirm the findings among COVID-19 patients in different conditions, such as relative to age and cardiac risk factors.

In summary, we observed moderate correlations of D-dimer with NLR and IL-6 levels. These findings implicate further studies of early anti-coagulant treatment with cytokine storm in COVID-19 patients and the possibility of preventing deleterious cytokine damage in patients. This study, combined with previous observations of coagulopathy in COVID-19-infected patients (16, 21–23), will help the medical field to develop an effective clinical strategy. Anti-coagulant treatment could represent a novel preventive treatment strategy to block a severe clinical cytokine storm in COVID-19 patients with moderate or mild condition.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of first affiliated hospital, Bengbu Medical College. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HG, WL, JL (6th Author), FL, ZZ, JL (11th Author), YZ, JG, GL, and LW collected the data. HG, YS, and JL (11th Author) analyzed the data, processed statistics, wrote the manuscript, and revised the manuscript. HG, JL (11th Author), and FW supervised the study. All authors contributed to the article and approved submission.

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of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors thank the patients who provided their data to this study.

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Predictive Factor for COVID-19 Worsening: Insights for High-Sensitivity Troponin and D-Dimer and Correlation With Right Ventricular Afterload

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Background: Coronavirus disease 2019 (COVID-19) has been associated with cardiovascular complications and coagulation disorders.

Objectives: To explore clinical and biological parameters of COVID-19 patients with hospitalization criteria that could predict referral to intensive care unit (ICU).

Methods: Analyzing the clinical and biological profiles of COVID-19 patients at admission.

Results: Among 99 consecutive patients that fulfilled criteria for hospitalization, 48 were hospitalized in the medicine department, 21 were first admitted to the medicine ward department and referred later to ICU, and 30 were directly admitted to ICU from the emergency department. At admission, patients requiring ICU were more likely to have lymphopenia, decreased SpO_2, a D-dimer level above 1,000 ng/mL, and a higher high-sensitivity cardiac troponin (Hs-cTnI) level. A receiver operating characteristic curve analysis identified Hs-cTnI above 9.75 pg/mL as the best predictive criteria for ICU referral [area under the curve (AUC), 86.4; 95% CI, 76.6–96.2]. This cutoff for Hs-cTnI was confirmed in univariate [odds ratio (OR), 22.8; 95% CI, 6.0–116.2] and multivariate

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analysis after adjustment for D-dimer level (adjusted OR, 20.85; 95% CI, 4.76–128.4). Transthoracic echocardiography parameters subsequently measured in 72 patients showed an increased right ventricular (RV) afterload correlated with Hs-cTnI ($r = 0.42$, $p = 0.010$) and D-dimer ($r = 0.18$, $p = 0.047$).

Conclusion: Hs-cTnI appears to be the best relevant predictive factor for referring COVID-19 patients to ICU. This result associated with the correlation of D-dimer with RV dilatation probably reflects a myocardial injury due to an increased RV wall tension. This reinforces the hypothesis of a COVID-19-associated microvascular thrombosis inducing a higher RV afterload.

Keywords: COVID-19, troponin, D-dimer (DD), echocardiograghy, thrombosis, right ventricle

1. WHAT IS KNOWN ABOUT THIS TOPIC?

COVID-19 is associated with cardiovascular complications and coagulation disorders.
Predictive markers of severity and intensive care unit (ICU) referral are required upon hospital admission.

2. WHAT DOES THIS PAPER ADD?

D-Dimer and high sensitivity cardiac troponin (Hs-cTnI) at hospital admission are prognosis biomarkers for ICU referral.
Hs-cTnI appears the best relevant predictive factor for ICU referral in COVID-19 patients.
D-Dimer and Hs-cTnI elevation are correlated with the increase of right ventricular afterload observed in COVID-19.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can be asymptomatic or lead to the coronavirus disease 2019 (COVID-19), which has not only a very large pattern of respiratory manifestations but also other non-specific symptoms including fever, headache, hemoptysis, nausea, vomiting, and diarrhea also previously described in other coronavirus infections (1, 2). In terms of respiratory symptoms, COVID-19 is characterized by a large spectrum of infectious signs from dry cough and pulmonary edema to acute respiratory distress syndrome (ARDS), requiring hospitalization in intensive care unit (ICU) and leading to death in the most severe cases (2). While patients with cardiovascular comorbidities are described to have the higher mortality rate in published data (3), it is however challenging to provide a risk stratification of disease progression at admission for COVID-19 patients. Respiratory condition can worsen rapidly and is currently difficult to predict. From the very first consultation of the patient, biological markers could help to predict COVID-19 systemic consequences that would require ICU referral.

Several biomarkers of COVID-19 have been associated with the disease severity and progression. COVID-19-associated coagulopathy was found more frequent in fatal COVID-19 cases, and a high D-dimer level has been associated with poor prognosis and in-hospital mortality (4–6). The hypothesis of the presence of microthrombi in lungs and kidneys was suggested after autopsy case series (7–9). In COVID-19, microvascular thrombosis is observed during an inflammatory storm and could participate in damaging capillary endothelium and disrupting the thrombo-protective state of endothelial cells (10–12). SARS-CoV-2 has been shown to infect blood vessels and induce vascular damage *in vitro* and *in vivo* (13–15). SARS-CoV-2 can infect cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is ubiquitous but largely expressed in endothelial cells (16). Endotheliitis could be at the origin of impaired microcirculatory function affecting the lungs and kidneys (15, 17, 18). Furthermore, we previously described the endothelial lesion in patients with hospitalization criteria as a marker of COVID-19 severity at hospital admission (19). Acute myocardial injuries were also largely reported, such as numerous acute coronary syndromes or myocarditis (20). Cardiac troponin is increased in the case of community-acquired pneumonia, in the context of myocardial oxygen supply–demand mismatch and supply (21), and seems to correlate with the severity of respiratory impairment. Yet, few data are currently available on troponin level at the time of COVID-19 diagnosis (20, 22–24). Mechanisms of myocardial and tissue injury as well as coagulopathy associated with COVID-19 could be linked to the cytokine storm (25).

In this study, we aimed at identifying, in COVID-19 patients with criteria for hospitalization, biological and clinical markers at admission that could predict future referral in ICU and help anticipating a worsening of the patient's condition. We also investigated correlations between biological markers and cardiac function, assessed by ultrasound.

METHODS

Study Design and Population

This study is an observational cohort study conducted at the Georges Pompidou European Hospital in Paris, France. We prospectively included consecutive patients with confirmed SARS-CoV-2 infection. Inclusion criteria were patients over 18 years of age, with COVID-19, who consulted the emergency department with hospitalization criteria. Primary endpoint was ICU transfer, according to the usual criteria of ICU requirement, described in **Table 1**, and was kept unchanged

TABLE 1 | Criteria to admit COVID-19 patients to ICU.

ICU referral criteria for COVID-19 patients
Respiratory failure requiring mechanical ventilation at 6–8 L/min of oxygen to maintain SpO_2 >90–92%
and/or signs of respiratory distress (≧30 breaths/min), thoraco-abdominal swaying, inspiratory depression of the suprasternal trough
and/or other associated failure (s): loss of consciousness with Glasgow Coma Scale <12; systolic arterial pressure <90 mmHg, signs of peripheral hypoperfusion

ICU, intensive care unit; SpO_2, pulse oximetric saturation.

throughout the study. All patients were confirmed with SARS-CoV-2 infection by nasopharyngeal swabs. For each patient included, clinical evaluation and computed tomography (CT) scan and biological evaluation were performed. For all patients, baseline characteristics (demographics, treatment, clinical examination, cardiovascular risk factors, and body mass index) and biological data were retrieved from the medical records using a standardized data collection.

Routine Blood Examinations

All samples were collected in ethylenediaminetetraacetic acid (EDTA), sodium heparin, and 0.129 M trisodium citrate tubes (9NC BD Vacutainer©). Routine lab tests included complete blood count, creatinine, C-reactive protein (CRP), and high-sensitivity cardiac troponin (Hs-cTnI, Beckman) on a DXI analyzer (26, 27). Coagulation tests were prothrombin time (PT) ratio, fibrinogen, and soluble fibrin monomer level (STA-Liatest FM®; Diagnostica Stago©) explored on a STA-R® Max coagulometer (Stago©) as previously described (28). D-Dimer concentrations were determined using the Vidas D-Dimer assay (BioMérieux©) according to the manufacturer's instructions.

Transthoracic Cardiac Ultrasound and ICU Respiratory Parameters Evaluation

Cardiac ultrasound was performed on 72 additional consecutive COVID-19 patients, in the medicine department ($n = 32$) and ICU ($n = 40$). Transthoracic echocardiography (TTE) was performed using commercially available equipment CX50®, S5-1 probe (1–5 MHz; 80 elements) (Philips Medical Systems©, Andover, Massachusetts), according to the guidelines of the American Society of Echocardiography, during the first 24 h after hospital admission (29). At the time of echocardiographic examination, heart rate (HR), systolic, diastolic, and mean blood pressure were recorded. The examination included standard parasternal view to assess the size of the left ventricle (LV) [LV end diastolic diameter, (LVEDD); LV end systolic diameter (LVESD)]. LV ejection fraction was determined using the Simpson's method. Velocity time integral (VTI, cm) of the LV outflow tract and cardiac output were measured on an apical five-chamber view. Using the apical four-chamber view, mitral inflow was recorded by pulse-wave Doppler. We assessed the early diastole (E, cm/s) and the atrial contraction (A, cm/s). Early diastole (e′, cm/s) velocity of the lateral mitral annulus was measured by tissue Doppler imaging. We then calculated the E/A and E/e′ ratios. Using the same view, we carried out an evaluation of the right ventricle (RV): tricuspid annular plane systolic excursion (TAPSE) with TM mode, S wave velocity with tissue Doppler imaging, peak systolic tricuspid insufficiency velocity, and ratio of basal diameters of the right and left ventricle (RV/LV). The trans-tricuspid pressure difference was estimated from the peak velocity of tricuspid regurgitant jet (TR Vmax) by applying the simplified Bernoulli equation. The systolic pulmonary arterial pressure (sPAP) was then derived by adding estimated right atrium pressure based on the inspiratory changes in the dimension of inferior vena cava (30).

We also collected respiratory characteristics [FiO_2, positive end-expiratory pressure (PEEP), plateau pressure (Pplat), respiratory system compliance] and hemodynamic parameters [mean arterial pressure (MAP), HR, catecholamine dose] at the time of TTE assessment. The blood gas samples were collected just before the TTE.

Statistical Analysis

Continuous data were expressed as median [interquartile range (IQR)] and categorical data as proportion. Patients were compared according to patients' care pathway divided into three groups: patient hospitalized in the medical department, patients hospitalized in the medical department and then referred to ICU following respiratory worsening, and patients directly admitted to the ICU. In this univariate analysis, continuous variables were compared using Kruskal–Wallis test, and categorical variables were compared using Cochran–Armitage test for trend (multiple group). Patients were also compared according to the Hs-cTnI at admission. In this univariate analysis, we determined the differences in median using the unpaired *t*-test (Mann–Whitney *U* test) for continuous variable, and differences in proportions were assessed with the chi-square test or Fisher's exact test if necessary. We generated receiver operating characteristics (ROC) curves with four different regression models that included variables with significant difference in the univariate analysis (31, 32). Models included (i) plasma level of D-dimer (over a cutoff value of 1,000 ng/mL only), (ii) the Hs-cTnI only, (iii) the Hs-cTnI adjusted on gender, the presence of pneumonia on CT scan and the plasma level of D-dimer (over a cutoff value of 1,000 ng/mL), and (iv) the gender, the presence of pneumonia on CT scan, and the plasma level of D-dimer (over a cutoff value of 1,000 ng/mL). The four models helped assess the extent to which the level of D-dimer and Hs-cTnI influenced the predictability of hospitalization in ICU. We calculated the area under the curve (AUC) for the different logistic regression model (32, 33). We used logistic regression to determine whether the level of D-dimer (as a categorical dependent variable dichotomized according to the cutoff of 1,000 ng/mL) and the level of Hs-cTnI (as a categorical dependent variable dichotomized according to the cutoff of 9.75 pg/mL) were associated with the ICU referral (34, 35). The model included only these two variables (D-dimer and Hs-cTnI), and to take into account any potential interaction, we performed the model with and without interaction term.

TABLE 2 | Demographic and clinical characteristics of patients at admission according to the addressed department (medical or ICU).

	Medicine patients ($n = 48$)	Medicine then ICU patients ($n = 21$)	ICU patients ($n = 30$)	*p*-Value
Male sex—*n* (%)	31 (64.6)	17 (81.0)	26 (86.7)	0.070
Age—years, median (IQR)	62.5 (50.8–80.0)	67.0 (55.0–75.0)	60.0 (55.0–69.8)	0.764
BMI—kg/m^2, median (IQR)	26.3 (24.7, 29.3)	26.7 (24.9, 28.1)	27.3 (25.0–30.5)	0.810
Time from illness onset to hospital admission—days	4.5 (3.0–7.0)	7.0 (4.0–8.0)	7.0 (4.0–9.0)	0.073
CV risk factors, *n* (%)				
Hypertension	19 (39.6)	11 (52.4)	16 (53.3)	0.624
Dyslipidemia	11 (22.9)	5 (3.8)	9 (30.0)	0.771
Diabetes	6 (12.5)	8 (38.1)	9 (30.0)	0.062
Sedentary lifestyle	4 (8.3)	0 (0.0)	2 (6.7)	0.715
Chronic kidney disease	4 (8.3)	4 (19.0)	3 (10.0)	0.416
Medical history, *n* (%)				
Cancer	4 (8.3)	1 (4.8)	1 (3.3)	0.641
Coronary heart disease	4 (8.3)	1 (4.8)	5 (16.7)	0.002
Stroke	3 (6.2)	2 (9.5)	2 (6.7)	0.883
Treatments, *n* (%)				
Statins	11 (22.9)	5 (23.8)	9 (30.0)	0.771
Oral antidiabetic agents	5 (10.4)	6 (28.6)	8 (26.7)	0.098
Insulin	2 (4.2)	4 (19.0)	3 (10.0)	0.138
β-blocker	5 (10.4)	3 (14.3)	5 (16.7)	0.718
Calcium channel blockers	8 (16.7)	6 (28.6)	5 (16.7)	0.470
ACEi or ARBs	13 (27.1)	6 (28.6)	12 (40.0)	0.466
ARBs	6 (12.5)	3 (14.3)	5 (16.7)	0.864
Diuretics	4 (8.3)	4 (19.0)	4 (13.3)	0.442
Central acting agent	1 (2.1)	0 (0.0)	0 (0.0)	0.585
Clinical features, *n* (%)				
Fever	44 (91.7)	20 (95.2)	28 (93.3)	0.863
Headache	10 (20.8)	8 (38.1)	15 (50.0)	0.089
Cough	33 (68.8)	19 (90.5)	25 (83.3)	0.268
Productive cough	6 (12.5)	1 (4.8)	2 (6.7)	0.505
Dyspnea	19 (39.6)	15 (71.4)	28 (93.3)	<0.001
Myalgia	14 (29.2)	6 (28.6)	12 (40.0)	0.559
Diarrhea	3 (6.2)	7 (33.3)	4 (13.3)	0.045
Pneumonia	32 (66.7)	19 (90.5)	29 (96.7)	0.002
ARDS	0 (0.0)	2 (9.5)	11 (36.7)	<0.001
SpO_2–%, median (IQR)	95.0 (92.5–96.0)	92.0 (90.0–96.0)	89.0 (84.0–92.0)	<0.001
Respiratory rate—breathes per min, median (IQR)	18.0 (16.0–20.0)	20.0 (16.0–25.0)	23.0 (21.0–32.0)	0.001
Pulse—beats per min, median (IQR)	87.0 (76.5–99.0)	88.0 (80.0–98.0)	97.0 (87.0–110.0)	0.060
Biological parameters, *n* (%)				
White blood cells—$\times 10^9$/L, median (IQR)	5.85 (4.52–7.03)	4.60 (4.20–6.90)	7.20 (5.10–11.10)	0.034
Hemoglobin—g/L, median (IQR)	130.5 (111.5–148.0)	140.0 (129.0–151.0)	130.0 (119.0–140.0)	0.213
Platelet count—$\times 10^9$/L, median (IQR)	171.5 (149.8–228.0)	147.0 (117.0–197.0)	179.0 (138.0, 247.0)	0.042
Polynuclear neutrophils—$\times 10^9$/L, median (IQR)	3.92 (2.94–5.36)	3.74 (2.55–5.90)	6.14 (3.99–10.00)	0.003
Lymphocytes—$\times 10^9$/L, median (IQR)	0.97 (0.76–1.35)	0.74 (0.63–1.01)	0.60 (0.44–0.95)	0.002

(Continued)

TABLE 2 | Continued

	Medicine patients ($n = 48$)	Medicine then ICU patients ($n = 21$)	ICU patients ($n = 30$)	*p*-Value
Monocytes—$\times 10^9$/L, median (IQR)	0.48 (0.35–0.66)	0.34 (0.24–0.44)	0.34 (0.23–0.53)	0.005
CRP—mg/L, median (IQR)	64.8 (14.3–100.4)	104.0 (57.1–162.0)	164.0 (105.5–209.8)	<0.001
Plasma creatinine—μmol/L, median (IQR)	72.0 (60.0, 89.0)	89.0 (80.0–119.0)	101.0 (75.5–179.3)	0.002
Hs-TNI—pg/mL, median (IQR)	5.6 (4.3–11.3)	20.0 (10.5–35.5)	26.0 (18.0–95.0)	<0.001
PT ratio, median (IQR)	0.96 (0.91–1.03)	0.94 (0.91–1.00)	0.86 (0.77–0.96)	0.009
Fibrinogen—g/L, median (IQR)	5.1 (4.7–5.8)	5.7 (5.6–6.5)	6.5 95.8–7.3)	<0.001
D-dimer ≥1,000 ng/mL—***n*** (%)	15 (31.2)	11 (52.4)	21 (70.0)	0.013
D-dimer—ng/mL, median (IQR)	840 (570–1,462)	1,455 (630–2,003)	1,358 (957–2,122)	0.060
Fibrin monomers—μg/mL, median (IQR)	<7.0 (<7.0–<7.0)	<7.0 (<7.0–<7.0)	<7.0 (<7.0–<7.0)	0.876

ICU, intensive care unit; BMI, body mass index; CV, cardiovascular; ACEi, angiotensin conversion enzyme inhibitor; ARB-2, antagonist of angiotensin 2 receptor blocker; SpO_2, pulse oximetric saturation; ARDS, acute respiratory distress syndrome; IQR, interquartile range; CRP for C-reactive protein; Hs-TnI for ultrasensitive troponin I; PT, prothrombin time. p trend between three groups.

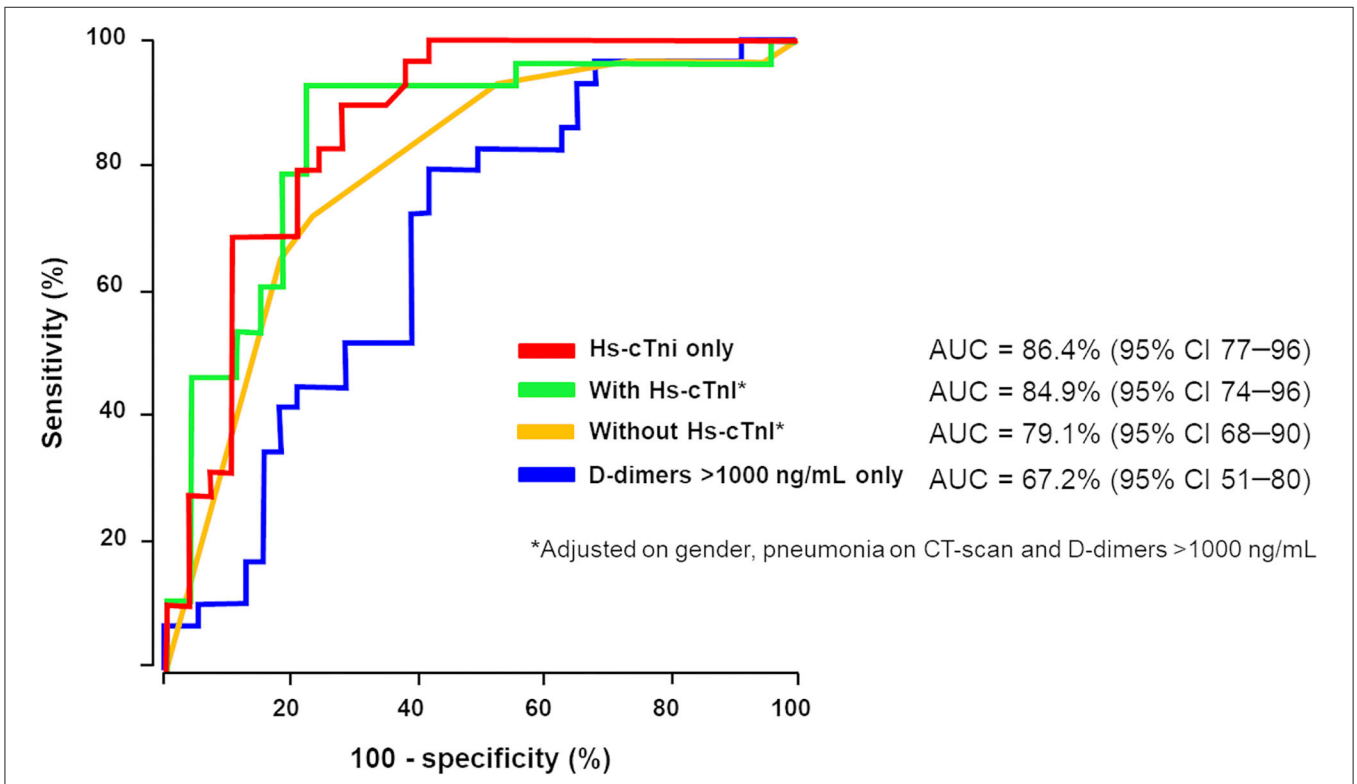

FIGURE 1 | D-Dimer and high-sensitivity cardiac troponin (Hs-cTnI) involvement in intensive care unit (ICU) referral. We used different D-dimer level cutoffs (>1,000 ng/mL, >2,000 ng/mL, >3,000 ng/mL) as potential prognostic criteria for ICU referral. Receiver operating characteristics (ROC) curve analysis associating D-dimer above 1,000 ng/mL, gender and pneumonia at CT scan for ICU transfer (in yellow) increases area under the curve (AUC) in contrast to D-dimer ≥1,000 ng/mL alone (AUC, 79.1; 95% CI, 68–90, $p = 0.04$). Addition of Hs-cTnI to this model (in green) allowed reaching AUC of 84.9 (95% CI, 74–96, $p = 0.03$). Hs-cTnI alone was the best predictive ROC curve (in red) for ICU outcome with AUC of 86.4 (95% CI, 77–96).

The correlation between biological parameters (Hs-cTnI and D-dimer) at hospital admission and ultrasound characteristics of patients who were hospitalized in ICU with invasive mechanical ventilation was assessed using the Kendall coefficient correlation test. All analyses were two-sided and a $p < 0.05$ was considered statistically significant. Statistical analysis was performed using R studio software (R© Development Core Team, 2019).

RESULTS

D-Dimer and Hs-cTnI Levels at Hospital Admission Are Discriminant Biomarkers to Predict ICU Referral

The cohort study was composed of 99 consecutive patients who presented to the emergency department and were diagnosed with COVID-19 in March and April 2020. They were divided into three groups: patients hospitalized in the medical department ($n = 48$), patients first hospitalized in the medicine department (mean of 3.0 ± 1.4 days) then referred to ICU due to respiratory degradation ($n = 21$), and patients admitted to ICU after admission to the emergency department ($n = 30$).

These three groups were strictly comparable in terms of age, body mass index, cardiovascular risk factors, treatments, and time from illness onset to hospitalization (**Table 2**). However, COVID-19 patients directly admitted to ICU had more often history of coronary heart disease and were more likely to have dyspnea at admission ($p < 0.001$), decreased SpO_2 ($p < 0.001$), pneumonia on the CT scan ($p = 0.002$), ARDS ($p < 0.001$), and increased respiratory rate–breath per minute ($p < 0.001$). In terms of biological features, patients directly requiring ICU admission and those referred to ICU after conventional hospitalization had a significantly higher white blood cell count and granulocytes count (respectively, $p = 0.03$ and 0.003) with more severe lymphopenia and monocytopenia (respectively, $p = 0.002$ and 0.005) than patients hospitalized in the medicine department. Regarding coagulation disorders, 70% of COVID-19 patients admitted to ICU had D-dimer level above 1,000 ng/mL at hospital admission ($p = 0.0013$). The PT ratio was significantly different between groups; however, it remained within normal range. In the whole COVID-19 population, fibrin monomers were negative and associated with hyperfibrinogenemia and without thrombocytopenia. These results were not in favor of a COVID-19-associated disseminated intravascular coagulation (DIC). Lastly, COVID-19 patients admitted to ICU had a significantly higher CRP level ($p < 0.001$), plasma creatinine ($p = 0.002$), and Hs-cTnI ($p < 0.001$). No correlation was found between Hs-cTnI and D-dimer ($p = 0.82$), Hs-cTnI and creatinine ($p = 0.27$), or Hs-cTnI and SpO_2 ($p = 0.13$), while a significant association was found between Hs-cTnI and CRP ($p = 0.001$).

Hs-cTnI Level at Entrance Is the Most Relevant Biomarker to Predict ICU Referral

In the context of coagulopathy and myocardial injury during COVID-19 and given the observation that D-dimer and Hs-cTnI levels at admission were associated with ICU admission, a ROC curve analysis was constructed using these biomarkers (**Figure 1**). We used different D-dimer level cutoffs (>1,000, >2,000, and >3,000 ng/mL) as potential prognostic criteria for ICU referral. For the cutoff of >1,000 ng/mL, the ROC curve (AUC, 67.2; 95% CI, 51.4–80.2) yielded a sensitivity of 72.4% (95% CI, 50.0–86.0), a specificity of 60.0% (95% CI, 43.0–75.0), a positive predictive value (PPV) of 58.6% (95% CI, 40.0–74.0), and a negative predictive value (NPV) of 74.2% (95% CI, 55.0–87.0%, **Table 3**). None of the other cutoffs had better prognostic values (**Table 3**). The D-dimer cutoff of 1,000 ng/mL as a predictive value for ICU referral was improved in a ROC curve analysis, when associated with gender and pneumonia at CT scan (AUC, 79.1; 95% CI, 68.5–89.7, $p = 0.04$). The addition of Hs-cTnI to this model allowed reaching an AUC of 84.9 (95% CI, 73.9–95.9, $p = 0.03$). Furthermore, Hs-cTnI alone was the best predictor for ICU outcome with AUC of 86.4 (95% CI, 76.6–96.2). ROC curve identified a cutoff at 9.75 pg/mL yielding a high sensitivity of 89.6% (95% CI, 71.0–97.0), a good specificity of 72.4% (95% CI, 52.0–86.0), a high PPV of 76.5% (95% CI, 58.0–88.0), and a high NPV of 87.5% (95% CI, 66.0–96.0). None of the other Hs-cTnI cutoffs currently used for acute myocardial infarction [11.6 and 19.8 pg/mL, respectively, for men and women; cutoff detection for acute myocardial injury defined as an elevated Hs-cTnI value above the 99th percentile upper reference limit (27)] had a prognostic value better than 9.75 pg/mL (**Table 4**).

Table 5 confirms the link between Hs-cTnI and ICU referral using a logistic regression model with the cutoff of 9.75 pg/mL for Hs-cTnI (OR, 22.8; 95% CI, 6.0–116.2, $p < 0.001$). Strikingly, when adjusted to D-dimer level, the adjusted OR of 20.85 (95% CI, 4.76–128.4, $p < 0.001$) was not better. Inversely, when a logistic regression model used the cutoff of 1,000 ng/mL for D-dimer, the association between D-dimer and ICU outcome (OR, 4.02; 95% CI, 1.46–11.93, $p = 0.009$) did not remain

TABLE 3 | Evaluation of various D-dimer cutoffs at admission related to ICU referral.

D-dimer involvement in ICU referral						
Cutoff	**1,000 ng/mL**		**2,000 ng/mL**		**3,000 ng/mL**	
		95% CI		**95% CI**		**95% CI**
Sensitivity	72.4%	52–86	34.0%	18–54	10.0%	20–28
Specificity	60%	43–75	84.2%	68–93	86.3%	71.0–95
Positive predictive value	58.6%	40–74	62.5%	35–83	37.5%	10–74
Negative predictive value	74.2%	55–87	62.7%	48–75	55.9%	42–68

ICU, intensive care unit.

TABLE 4 | Various Hs-cTnI cutoffs at admission related to ICU referral.

Hs-cTnI involvement in ICU referral						
	Cutoff: 9.75 pg/mL		**Cutoff: 11.6 pg/mL**		**Cutoff: 19.8 pg/mL**	
		95% CI		95% CI		95% CI
Sensitivity	89.6%	71–97	82.7%	63–93	68.0%	49–84
Specificity	72.4%	52–86	75.8%	57–89	82.0%	63–93
Positive predictive value	76.5%	58–88	77.4%	58–89	80.0%	58–92
Negative predictive value	87.5%	66–96	81.5%	64–92	72.0%	54–86

Hs-cTnI, high sensitivity cardiac troponin; ICU, intensive care unit.

TABLE 5 | Logistic regression model evaluating D-dimer >1,000 ng/mL and high-sensitivity troponin level in ICU referral and mortality.

Logistic regression model with ICU referral as the outcome				
		OR (univariate) 95% CI	**OR (bivariate) 95% CI**	**OR (bivariate with interaction term) 95% CI**
D-dimer—ng/mL	<1,000	–	–	–
	>1,000	4.02 (1.46–11.93, $p = 0.009$)	1.34 (0.25–5.88, $p = 0.706$)	1.41 (0.06–19.35, $p = 0.760$)
Hs-TnI—pg/mL	<9.75	–	–	–
	>9.75	22.75 (6.03–116.17, p < 0.001)	20.85 (4.76–128.40, $p < 0.001$)	21.50 (3.07-271.12, $p = 0.005$)
Interaction term between Hs-TnI and D-dimer				0.84 (0.03–30.50, $p = 0.918$)
Metrics of the model	C-statistic		0.825	0.825
	AIC		57.3	59.4
Logistic regression model with mortality as the outcome				
D-dimer—ng/mL	<1,000	–	–	–
	>1,000	3.22 (1.17–9.94, $p = 0.030$)	1.49 (0.44–5.22, $p = 0.521$)	2.71 (0.10–75.26, $p = 0.500$)
Hs-TnI—pg/mL	<9.75	–	–	–
	>9.75	9.50 (2.44–63.36, $p = 0.004$	8.46 (1.99–59.23, $p = 0.010$)	11.87 (1.59–247.68, $p = 0.035$)
Interaction term between Hs-TnI and D-dimer				0.49 (0.01–16.54, $p = 0.657$)
Metrics of the model	C-statistic		0.730	0.725
	AIC		80.3	82

The model included only two variables (D-dimer and Hs-cTnI) with and without interaction term.
Hs-cTnI, high-sensitivity cardiac troponin; ICU, intensive care unit; OR, odds ratio; C-statistic, concordance statistic; AIC, Akaike information criterion.

significant when adjusted to Hs-cTnI (OR, 1.34; 95% CI, 0.3–5.9, $p = 0.7$), confirming the higher specificity of Hs-cTnI in predicting ICU referral in hospitalized patients. The addition of the interaction term between D-dimer and Hs-cTnI did not change the associations observed. To confirm the relevance of this proposed Hs-cTnI cutoff of 9.75 pg/mL, we compared clinical and biological results in the study population and found that patients with Hs-cTnI above 9.75 pg/mL were older ($p = 0.01$) and more likely to get a β-blocker prescription ($p = 0.034$), angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) therapy (respectively, $p = 0.005$ for ACEi or ARBs and $p = 0.021$ for ARBs alone), or a history of coronary heart disease ($p = 0.016$) (**Supplementary Table 3**). Lastly, we evaluated the link between Hs-cTnI and mortality among the same patients. The ROC curve identified a cutoff at 10.75 pg/mL yielding a sensitivity of 42.6% (95% CI, 29.5–56.8) and a specificity of 100% (95% CI, 80.7–100). We will confirm the relevance of an Hs-cTnI threshold of 9.75 pg/mL in a prospective study.

D-Dimer and Hs-cTnI Levels Are Related to Vascular Obstruction and Increased Right Ventricular Afterload on Transthoracic Cardiac Ultrasound Evaluation

We explored the cardiac function by TTE of 72 consecutive patients tested positive for COVID-19, admitted in April 2020, 32 (44%) patients in the medicine department, and 40 (56%) patients in ICU. Demographic data and respiratory function are reported in **Supplementary Tables 1, 2**. Regarding patients in ICU, at the time of TTE assessment, median PEEP was 13 cmH_2O (5–16), median Pplat was 26 cmH_2O (19–28), median respiratory system compliance was 36.30 (mL/cmH_2O) (31.1–45.9), and FiO_2 50% (44–100). Regarding hemodynamic parameters median MAP was 70 mmHg (60–78), median HR was 105/min (85–116), while median epinephrine dose was 0.0 mg/h (0.0–0.9). Most patients had respiratory acidosis with median pH 7.28 (7.20–7.34), median $PaCO_2$ of 53 mmHg (42–59), median PaO_2 of 85 mmHg (68–100), and median lactate of 1.45 mmol/L (1.1–2.0).

As described in **Supplementary Table 4**, we found no change in the LV ultrasound parameters. No significant abnormalities in the LV geometry [LVEDD at 44.0 (41.9–50) mm or LVESD at 31.0 (26.4–37) mm], LV ejection fraction [60 (55–65)%], or LV filling pressures [E/A ratio at 0.9 (0.7–1.4)], with a median E wave at 75 (59–85.2) cm/s and E/lateral e′ ratio at 6.2 (4.9–8.3), were indeed observed. Regarding the relationship between biological parameters at hospital admission and ultrasound characteristics, Hs-cTnI showed a significant but weak correlation with the LV ejection fraction ($r = -0.196$, $p = 0.039$) and no correlation with the E/e′ ratio ($p = 0.157$). D-Dimer did not correlate with these parameters. We did not find any acute systolic dysfunction associated with COVID-19. Hs-cTnI elevation corresponded rather to a higher rise in heart disease with underlying low LVEF.

Concerning the RV, we found an initial dilatation of the RV diameter [median diameter of 37.8 (33.0–43.3) mm], with an RV/LV ratio of 0.8 (0.7–0.9), without dilatation of the LV. RV function was maintained [s′ tissue Doppler imaging (TDI) at 13.0 (11.5–16.0) cm/s; tricuspid annular plane systolic excursion

TABLE 6 | Correlations between biological markers and echocardiographic features.

Biomarker	TTE parameter	Correlation coefficient	*p*-Value
Left ventricle parameters			
Hs-cTnI	LVEF	−0.195	0.039
Hs-cTnI	E/e′ ratio	0.157	0.076
D-dimer	LVEF	0.027	0.770
D-dimer	E/e′ ratio	0.092	0.288
Right ventricle parameters			
Hs-cTnI	RV diameter	0.177	0.060
Hs-cTnI	RV/LV ratio	−0.028	0.765
Hs-cTnI	sPAP	0.425	0.010
Hs-cTnI	TR Vmax	0.380	0.010
Hs-cTnI	TAPSE	−0.236	0.007
Hs-cTnI	S wave (RV)	−0.133	0,329
D-dimer	RV diameter	0.234	0.012
D-dimer	RV/LV ratio	0.147	0.116
D-dimer	sPAP	0.178	0.047
D-dimer	TR Vmax	0.201	0.026
D-dimer	TAPSE	−0.181	0.035
D-dimer	s wave (RV)	0.161	0.251

Hs-cTnI, high-sensitivity cardiac troponin; LVEF, left ventricular ejection fraction; RV, right ventricle; LV, left ventricle; sPAP, systolic pulmonary arterial pressure; TAPSE, tricuspid annular plane systolic excursion; S wave (RV), positive systolic wave of the right ventricle using Tissue Doppler imaging.

(TAPSE) at 21.0 (17.8–23.6) mm]; the systolic pulmonary arterial pressure (sPAP) value remained above normal but without major elevation [31.6 (25.1–43.6) mmHg]. Regarding the ultrasound parameters of the RV, the strongest correlation was between Hs-cTnI level and sPAP ($r = 0.42$; $p = 0.01$, **Table 6**). Hs-cTnI was also correlated with an RV systolic dysfunction (correlation with TAPSE, $r = -0.24$; $p = -0.007$) but not with an RV dilatation (RV/LV ratio, $p = -0.765$). D-Dimer levels showed significant correlations with the same parameters, sPAP ($r = 0.18$, $p = 0.046$), TAPSE ($r = 0.18$, $p = 0.035$), and with the dilatation of the RV ($r = 0.23$, $p = 0.012$).

DISCUSSION

In this prospective single-center study, we reported that Hs-cTnI level at admission was the best biomarker to predict ICU transfer and respiratory severity in COVID-19 patients. Moreover, we evidenced the D-dimer involvement in the pathophysiology of COVID-19 and the correlation with a RV afterload, which allows us to confirm pulmonary vascular obstruction as a site of coagulopathy and a source of circulating D-dimer.

D-dimer increase has been widely reported during SARS-CoV-2 infection (2, 17, 36–38). It has been first associated with sepsis-induced coagulopathy and with DIC (37, 39). However, beside a high D-dimer level, the present study evidenced a low level of fibrin monomers, a high fibrinogen level, and no signs of thrombocytopenia, allowing us to exclude a DIC at admission. D-dimer may reflect the consequences of the COVID-19-associated coagulopathy (40, 41), as it probably participates in the respiratory disease through the development of capillary microthrombosis as observed in postmortem studies (42) and attributed to a vascular thickening or vascular congestion (14, 43–45). Moreover, another pulmonary vascular issue in COVID-19 is related to a high incidence of pulmonary embolism (PE) (46, 47) whose exact association still needs to be determined. However, our results show a low prognostic value of D-dimer levels on ICU referral when taken alone. In the same line, this finding could be similar to the use of D-dimer in PE, which has a low predictive performance when using absolute values and needs an age-adjusted cutoff. Interestingly, we previously observed that the of D-dimer level <500 ng/mL associated with female gender and absence of pneumonia at CT scan as potential exclusion criteria for COVID-19 diagnosis (17). Increase in D-dimer level and its evolution is probably a better reflect of COVID-19-associated coagulopathy involvement and progression that might help to choose between a prophylactic or a therapeutic anticoagulation strategy. This hypothesis needs to be confirmed in ongoing prospective randomized clinical trials.

The major finding of the present study is the excellent prognostic value of Hs-cTnI level to predict respiratory worsening, ICU referral, and mortality. It is important to note that the proposed Hs-cTnI cutoff is lower than the thresholds used for myocarditis or myocardial infarction diagnosis (48). The observed elevation of Hs-cTnI may probably be more resulting from microcirculatory damage and myocardial oxygen supply–demand mismatch and supply than a primary pathology of the myocardium, as it was already observed in the case of community-acquired pneumonia (21, 49). In parallel with myocardial inflammation due to SARS-CoV-2 infection, myocardial damage could result from a RV wall tension due to an increased RV afterload with RV dilation and tricuspid valve insufficiency. Yet, the mechanism of troponin release from the myocardium is not fully understood, and various pathophysiological scenarios have been proposed (50). However, the higher the troponin, the higher the risk of ICU referral (51, 52), with no altered LV function probably underlining the burden of endotheliitis and microthrombotic processes in the outcome of patients with COVID-19.

In the present study, Hs-cTnI was clearly correlated with sPAP values as well as with RV longitudinal systolic dysfunction. These results suggest that an increase in pulmonary pressures secondary to capillary microthrombosis may be responsible for right ventricular myocardial distress, which leads to troponin release. This hypothesis is also supported by the results of Szekely et al. showing RV dysfunction at the forefront of cardiac damage associated with COVID-19 and correlated with troponin levels (53). Although troponin was the best prognostic marker of patient worsening, D-dimer levels also have a prognostic role, appearing to correspond to a causal process of pulmonary microthrombosis. Thus, we suggest here a kinetic study of cardiopulmonary-induced lesion in COVID-19 as proposed in **Figure 2**. Pulmonary endothelium forms a key part of the alveolar–capillary unit,

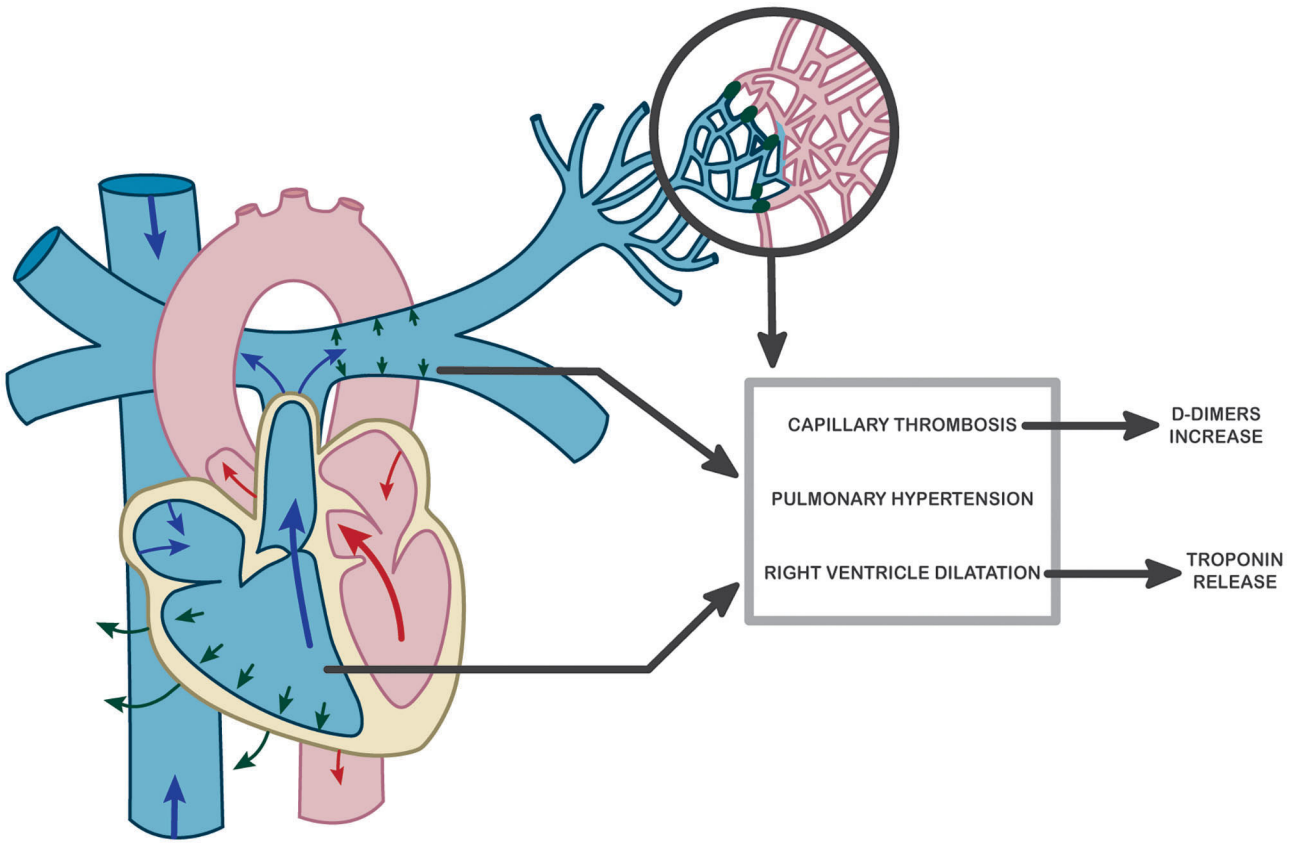

FIGURE 2 | Hypothesis of a potential pathophysiological mechanism explaining pulmonary and cardiac dysfunction in COVID-19 and resulting in troponin and D-dimer increase at admission to the hospital.

providing an interface for efficient gas exchange between the alveolar space and blood cells within lung capillaries. We previously described an early endothelial lesion that drives prognosis and ICU transfer of patients. This thrombo-inflammatory process in pulmonary vessels is probably the main actor of microthrombosis in lung capillaries (reflected by increased D-dimer) driving consequences in right ventricle. Thus, troponin increase is mainly reflecting RV afterload increase.

LIMITATIONS

This is a pilot study on the search for prognostic biomarkers of COVID-19, explaining this single-center work on a limited number of patients. We also do not present troponin kinetics, which were not systematically performed. A longer follow-up will be required to firmly define prognostic biomarkers of COVID-19 severity, and our results should be replicated in a multicenter study. Due to the small simple size and to respect the assumptions required to perform logistic regression, we were authorized to include in the model only these two variables (Hs-cTnI and D-dimer). For this reason, the influence of parameters such as gender or prior anticoagulant treatment (only three patients were treated for atrial fibrillation) could not be reasonably studied in this work. Thus, taking into account all of these limitations, the pathophysiological link between right ventricular afterload, cardiac troponin release, and COVID-19 is first and foremost a hypothesis requiring broader validation.

Despite these limitations, we believe that Hs-cTnI provides important information on the severity of COVID-19, like in pulmonary embolism, even with a cutoff value below the threshold usually used for acute coronary syndrome diagnosis (11.6 pg/mL for women and 19.8 pg/mL for men). Therefore, with the condition that each center adjusts its cutoff according to the intervariability of the method, Hs-cTnI could be considered as a relevant surrogate marker to avoid any delay in COVID-19 patient care and referral to ICU. Despite these limitations, we believe that, like in pulmonary embolism, Hs-cTnI, even at a low value below the threshold usually used (11.6 pg/mL for women and 19.8 pg/mL for men), provides important information on the severity of COVID-19.

In conclusion, it seems consistent to open the way for biomarkers in cardiovascular complications and coagulation disorders in COVID-19 patients at admission to the hospital. Further prospective studies should not only evaluate Hs-cTnI and D-dimer levels utility to predict admission to ICU but also evaluate their prognostic value during

follow-up and their relevance in respiratory and thrombotic related disorders.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de protection des personnes. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS, TM, and J-LD conceived and supervised the study. GG, J-LA, LK, NG, and BD monitored and analyzed the data. RC analyzed the data and supervised statistical analysis. All authors interpreted the data, drafted and revised the manuscript, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.586307/full#supplementary-material

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Thromboprofilaxys With Fondaparinux vs. Enoxaparin in Hospitalized COVID-19 Patients: A Multicenter Italian Observational Study

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Importance: The use of anticoagulant therapy with heparins decreased mortality in hospitalized patients with severe coronavirus disease 2019 (COVID-19). Even if enoxaparin and fondaparinux have the same clinical indication for venous thromboembolism (VTE) prevention; to date, there are no data about the use of fondaparinux in terms of safety, effectiveness, and impact on clinical prognosis among COVID-19 patients.

Objective: To evaluate the safety, effectiveness, and clinical impact of VTE prophylaxis with fondaparinux and enoxaparin among COVID-19 patients hospitalized in internal medicine units.

Design, Setting, and Participants: This was a retrospective multicenter observation study, including consecutive symptomatic patients with laboratory-proven COVID-19 admitted to internal medicine units of five Italian hospitals from 15th February to 15th March 2020.

Main Outcomes and Measures: The primary safety outcome was the composite of major bleeding and clinically relevant non-major bleeding; the primary effectiveness outcome was the composite of all events classified as pulmonary embolism and deep venous thrombosis. The secondary effectiveness outcome included acute respiratory distress syndrome and all-cause death.

Results: Among 120 COVID-19 patients enrolled in the study, 74 were taking enoxaparin (4,000 or 6,000 units/day) and 46 fondaparinux (2.5 units/day). No statistically significant difference in demographic and laboratory and clinical characteristics between the two groups has been shown. During a median follow-up of 32 (interquartile range: 14–51) days, the cumulative incidence rates of VTE and bleeding events on pharmacological

thromboprophylaxis with heparins were 19% and 8%, respectively. The incidence of both VTE (6.5 vs. 13.5%; $P = 0.36$) and bleeding events (6.5 vs. 4.1%; $P = 0.68$) did not show a significant difference between COVID-19 patients on fondaparinux compared with those on enoxaparin therapy. The regression model for the risk of outcome events according to different VTE prophylaxis drugs did not show significant differences.

Conclusions and Relevance: Although these results need confirmation by prospective studies including a larger population, our study provides preliminary evidence of a safe and efficacy use of fondaparinux for VTE prophylaxis in hospitalized COVID-19 patients.

Keywords: COVID-19, fondaparinux, enoxaparin, major bleedings, thromboprofylaxis, pulmonary embolism, deep venous thrombosis, acute respiratory distress syndrome

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 is a highly pathogenic human coronavirus recently recognized as the cause of the coronavirus disease 2019 (COVID-19), which spread rapidly from China to other countries, reaching devastating pandemic proportion (1, 2). The findings of increased D-dimer and fibrinogen levels in COVID-19 patients have prompted questions regarding the coexistence of venous thromboembolism (VTE), exacerbating ventilation–perfusion mismatch; in particular, pulmonary embolism (PE) seems to be prevalent (3). The complex interplay between inflammation and coagulation can significantly affect disease progression, leading to poor outcomes (4). The use of anticoagulant therapy with heparin was shown to decrease mortality in hospitalized patients with severe COVID-19 (5), probably because of its anti-inflammatory and antiviral proprieties (6). Even if enoxaparin and fondaparinux have the same clinical indication for VTE prevention (7), to date, there are no data about the use of fondaparinux in terms of safety, effectiveness, and impact on clinical prognosis among COVID-19 patients. Our study aimed to compare the safety and effectiveness of fondaparinux vs. enoxaparin in VTE prophylaxis among COVID-19 patients hospitalized in internal medicine units; moreover, the clinical impact in terms of acute respiratory distress syndrome (ARDS) development and in-hospital mortality has been evaluated.

MATERIALS AND METHODS

Patient Population

One hundred eighty-six consecutive symptomatic patients with laboratory-proven COVID-19 admitted to internal medicine units of five Italian hospitals from 15th February to 15th March 2020 were retrospectively evaluated for inclusion in the present study. Patients who were taking anticoagulant therapy for any medical reason before COVID-19 diagnosis (*n*: 34) or who experienced recent VTE (*n*: 5), major bleeding (MB), or clinically relevant non-major bleeding (CRNMB) within 30 days of hospital admission (*n*: 6) or with diagnosed VTE at admission (*n*: 21) were excluded from the study. We included hospitalized COVID-19 patients who underwent a VTE prophylaxis regimen according to the current international guidelines (7). During the hospitalization, the clinical status, laboratory examinations, instrumental ultrasound data, therapeutic regimens, the occurrence of PE, deep venous thrombosis (DVT), major (MB) and clinically relevant CRNMBs, acute severe respiratory distress syndrome (ARDS), and all-cause death were assessed.

Definitions

DVT was defined as a non-compressible venous segment or a substantial increase (4 mm or more) in the diameter of the thrombus during full compression in a previously abnormal segment on ultrasonography. PE was defined as a new intraluminal filling defect on spiral CT or pulmonary angiography or a new perfusion defect of at least 75% of a segment with corresponding normal ventilation (high probability), a new non-high-probability perfusion defect associated with DVT as documented by ultrasonography. MB was defined as fatal bleeding or symptomatic bleeding in a critical area or organ or bleeding causing a fall in hemoglobin level of $\geq$2 g/dl or more or leading to transfusion of two or more units of whole blood or red cells. CRNMB was defined as overt bleeding, not meeting the criteria for MB but requiring medical intervention (8). ARDS was defined according to the Berlin definition (9).

Outcomes

The primary safety outcome was the composite of MB and CRNMB; the primary effectiveness outcome was the composite of all events classified as PE and DVT. The secondary effectiveness outcome included ARDS and all-cause death.

Statistical Analysis

The Anderson–Darling test was used to analyze data normality. Continuous variables were reported using the median and interquartile intervals. Categorical variables were indicated as frequency counts and percentages. Differences in the two groups were evaluated using the two-tailed Fligner–Policello test for continuous data and Fisher's test for categorical variables. The effects of two different pharmacological treatments were tested in univariate analysis for the primary and secondary endpoints by using Cox proportional regressions analysis. Adjusted hazard ratios and 95% confidence intervals were estimated for each endpoint. A two-sided $P < 0.05$ was considered significant for

TABLE 1 | Demographic and laboratory and clinical characteristics of the study population.

Patients' characteristics	Enoxaparin group *N*: 74	Fondaparinux group *N*: 46	*P*-value
Males, *n* (%)	40 (54%)	24 (52.2%)	0.99
Age (years), median (IQR)	63 (55.3–73.76)	65 (53.6–77.7)	0.78
Hypertension, *n* (%)	36 (48.6%)	22 (47.8%)	0.92
Diabetes Mellitus, *n* (%)	15 (20.3%)	9 (19.6%)	0.88
COPD, *n* (%)	9 (12.2%)	6 (13%)	0.99
CAD, *n* (%)	13 (17.6%)	8 (17.3%)	0.99
CKD, *n* (%)	8 (10.8%)	4 (8.7%)	0.98
DCM, *n* (%)	12 (16.2%)	7 (15.2%)	0.91
PPS, median (IQR)	4 (4–5)	4.5 (4–6)	0.11
Previous stroke/TIA, *n* (%)	6 (8.1%)	3 (6.5%)	0.97
MPAP >40 mmHg, *n* (%)	2 (2.8%)	2 (4.3%)	0.99
D-dimer >500 mcg/dl at admission, *n* (%)	49 (66.2%)	34 (73.9%)	0.49
Fibrinogen >400 mcg/dl at admission, *n* (%)	55 (74.3%)	31 (67.4%)	0.54
Length of hospitalization (days), median (IQR)	31 (14–51)	34 (15–51)	0.90

CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DCM, dilated cardiomyopathy; PPS, Padua Prediction Score; IQR, interquartile range.

all tests. The net clinical benefit (NCB) was evaluated to obtain an integrated assessment of the anti-thromboembolic and pro-hemorrhagic effects of fondaparinux vs. enoxaparin. NCB was defined as the sum of incidence rates for VTE and bleeding events in the fondaparinux minus the sum of these rates in the enoxaparin group. Analyses were performed using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We selected 120 hospitalized COVID-19 patients who underwent a VTE prophylaxis regimen according to the current international guidelines. Seventy-four patients were on enoxaparin therapy (4,000 or 6,000 units/day); 46 patients were on fondaparinux (2.5 units/day). The use of fondaparinux and enoxaparin at standard (4,000 units/day) or high dose (6,000 units/day) was based on the patient's VTE risk, estimated by prediction score for risk of VTE (10). All patients showed a Padua Prediction Score for the risk of VTE $\geq$4. The median follow-up was 32 (interquartile range: 14–51) days. No statistically significant difference in demographic and laboratory and clinical characteristics between the two groups has been shown (**Table 1**).

Thirteen patients experienced incident VTE during the follow-up. The crude incidence rate of VTE was 13.5% (*n*: 10) in enoxaparin vs. 6.5% (*n*: 3) in the fondaparinux group ($P = 0.36$). Six patients experienced bleeding events during the follow-up.

The crude incidence rate of bleeding events was 4.1% (*n*: 3) in enoxaparin vs. 6.5% (*n*: 3) in the fondaparinux group ($P = 0.68$). Through these incidence rates, we found a positive NCB of fondaparinux over enoxaparin, equal to +4.6 (**Figure 1**).

Twenty-one patients developed ARDS during the follow-up. The crude incidence rate of ARDS was 18.9% (*n*: 14) in enoxaparin vs. 15.2% (*n*: 7) in the fondaparinux group ($P = 0.81$). Twelve patients died during the follow-up. The crude incidence rate of all-cause death was 9.5% (*n*: 7) in enoxaparin vs. 10.9% (*n*: 5) in the fondaparinux group ($P = 0.99$). The regression model analysis for pharmacological treatments related to the outcome events is shown in **Table 2**. The type of VTE prophylaxis drug did not result in a significantly increased risk of VTE, bleeding, ARDS, or in-hospital mortality among COVID-19 patients.

DISCUSSION

The high rate of coagulopathy and VTE among hospitalized patients with COVID-19 has been shown by several studies (11); however, little is still known about the potential association between antithrombotic therapies and COVID-19 clinical presentation or prognosis (12). The World Health Organization recommends the use of pharmacological prophylaxis with heparin for VTE prevention in COVID-19 patients[1]. The once-daily dosing regimen of low-molecular-weight heparins or fondaparinux should be preferred over unfractionated heparin to reduce personal protective equipment use and exposure of healthcare workers (13).

However, despite systematic thrombosis prophylaxis with low-molecular-weight heparins, the incidence of VTE among COVID-19 patients remains remarkably high and well comparable with that in other clinical settings characterized by disseminated intravascular coagulation (14). A recent meta-analysis by Fontana et al. (15) showed that the VTE risk ranges from 4.4 to 8.2% among the overall hospitalized patients with COVID-19; the highest risk, up to 53.8%, has been reported among critically ill patients with COVID-19 pneumonia hospitalized in an intensive care unit. Currently, little is still known about the VTE incidence among COVID-19 patients hospitalized in internal medicine units, and no data on the use of fondaparinux in this clinical setting have been reported yet.

Among our COVID-19 study population, a relatively high cumulative incidence of VTE events (10.8%) during pharmacological thromboprophylaxis with heparins has been showed; however, it was lower than those reported in previous studies from China (25%) and Europe (37%), which included only severe COVID-19 patients admitted to intensive care units (16, 17). The high incidence of VTE events despite the pharmacological thromboprophylaxis with heparins might be explained by the multifactorial genesis of COVID-19-associated coagulopathy. In particular, the excessive release of many inflammatory cytokines and chemokines, such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, and IL-8 (18), and the intense complement activation, with the deposition of the terminal complement complex C5b-9, C4d, and Mannan-binding lectin

[1]Available online at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected.

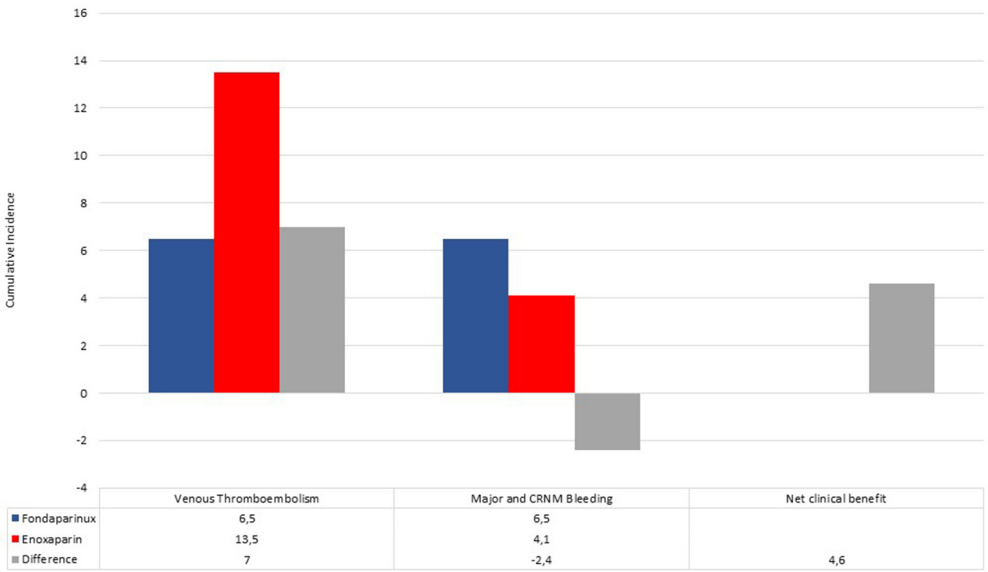

FIGURE 1 | Cumulative incidence of venous thromboembolism events, major and clinically relevant non-major bleedings in fondaparinux (blue bar) and enoxaparin (red bar) recipients. Differences (gray bar) between incidence rates were used to calculate the net clinical benefit (NCB).

TABLE 2 | Incidence and regression model for the risk of outcome events according to different VTE prophylaxis drugs.

Outcome events	Enoxaparin group *N*: 74	Fondaparinux group *N*: 46	Odds ratio (95% sCI)	*P*
VTE	10 (13.5%)	3 (6.5%)	2.25 (0.58–8.61)	0.24
DVT	5 (6.8%)	2 (4.3%)	1.59 (0.30–8.58)	0.54
PE	4 (5.4%)	0 (0%)	5.94 (0.31–112.87)	0.24
Bleedings	3 (4.1%)	3 (6.5%)	0.56 (0.11–2.91)	0.50
ARDS	14 (18.9%)	7 (15.2%)	1.30 (0.48–3.51)	0.60
All-cause dead	7 (9.5%)	5 (10.9%)	0.86 (0.25– 2.88)	0.80

VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; ARDS, acute respiratory distress syndrome.

serine protease 2 in the lungs (19), result in pulmonary microvascular thrombosis, vessel edema and hemorrhagic sequelae. A relatively high cumulative incidence of bleeding events (5%) has been reported, probably due to several prevalent cardiovascular comorbidities, such as diabetes, previous stroke, and hypertension, predisposing *per se* to bleeding events (20). The main finding of our study was that the incidence of both VTE and bleeding events did not show a significant difference between COVID-19 patients on fondaparinux compared with those on enoxaparin thromboprophylaxis; however, fondaparinux showed a higher net clinical benefit compared with enoxaparin. On the other hand, the use of fondaparinux did not show a statistically significant difference in terms of ARDS development and all-cause mortality compared with enoxaparin, with a numerically lower number of both ARDS and death events. These preliminary results support the hypothesis of safe and effective use of fondaparinux, compared with enoxaparin, among COVID-19 patients hospitalized in internal medicine units.

STUDY LIMITATIONS

The present study has several limitations: the retrospective nature of the analysis; the small number of enrolled patients; the short observational period limited to hospitalization. The study results are preliminary and hypothesis-generating; larger multicenter prospective studies are required to confirm our preliminary findings.

CONCLUSION

Our study supports the hypothesis of safe and efficacy use of fondaparinux for VTE prophylaxis in hospitalized COVID-19 patients, justified by a favorable net clinical benefit over enoxaparin.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The ethical committee of University of Campania approved the registry on anticoagulant treatments (751/2019).

AUTHOR CONTRIBUTIONS

PD performed study design. GCar, GV, and GCas performed statistical analysis. VR wrote the manuscript. AF, MI, and CL reviewed the draft. AB, MA, SM, AC, CS, FA, and PD selected patients for the study. All authors contributed to the article and approved the submitted version.

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frontiers in Medicine

BRIEF RESEARCH REPORT
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Systematic Screening for Deep Vein Thrombosis in Critically Ill Inpatients With COVID-19: Impact on the Incidence of Venous Thromboembolism

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Background: Several studies suggest an increased incidence of thrombosis in COVID-19 patients. However, evidence on how to prevent and even treat it is scarce. The aim of this study was to compare the cumulative incidence of venous thromboembolism (VTE) of two different methods for lower extremity deep vein thrombosis (LE-DVT) diagnosis: systematic vs. clinically guided complete compression venous ultrasonography (CCUS). We conducted a monocentric, prospective, open-label, non-randomized study. All consecutive patients admitted in three intensive care units (ICUs) of University Hospital of Toulouse for COVID-19 pneumonia were included: one performed systematic screening for LE-DVT, the others did not. The primary outcome was the 21-day cumulative incidence of VTE. The secondary end points were the 21-day cumulative incidences of major bleeding and death.

Results: Among the 78 patients included, 27 (34.6%) underwent systematic screening for DVT 7 ± 2 days after ICU admission. Thirty-two patients (41.0%) were diagnosed with VTE, with a 21-day cumulative incidence of 42.3% (95% CI, 31.4–55.2), without difference between screened and non-screened patients (hazard ratio 1.45, 95% CI, 0.72–2.93). In the screened group, the frequency of isolated DVT was higher (25.9 vs. 5.9%, p-value = 0.027), but the frequency of pulmonary embolism was not reduced (25.9 vs. 29.4%, p-value = 0.745). The 21-day cumulative incidences of major bleeding and death were 9.6% (95% CI, 4.7–19.2) and 10.3% (95% CI, 5.0–20.8), respectively, without difference between the two groups.

Conclusions: A systematic screening for DVT in patients hospitalized in ICU was not associated with a higher diagnosis of VTE or a reduced diagnosis of PE.

Keywords: COVID-19, venous thromboembolism, ultrasonography, deep vein thrombosis, intensive care unit

INTRODUCTION

Critically ill patients are at high risk for developing venous thromboembolism (VTE), with a frequency ranging from 5.1 to 15.5% despite the use of low-molecular-weight heparin (LMWH) thromboprophylaxis (1). Incidence is particularly high in patients diagnosed with sepsis, with a 30-day cumulative incidence of VTE of 12.5%, and 31% in patients with acute respiratory distress syndrome (ARDS) (2).

Early data suggested increased incidence of thrombosis in COVID-19 patients, particularly in critically ill patients, and have been confirmed since. In a multicentric study of 184 patients hospitalized in intensive care unit (ICU) with COVID-19 pneumonia, the adjusted cumulative incidence of thrombotic complication was 49% [95% confidence interval (95% CI), 41–57], with a majority of pulmonary embolism (PE) (3). In another monocentric study of 107 ICU patients with COVID-19, the cumulative incidence of PE at 15 days of admission was 20.4% (95% CI, 13.1–28.7), and frequency of PE was twice as high as that of influenza ICU patients admitted the year before (4). In a propensity score matching analysis, COVID-19 patients with ARDS developed more PE than non-COVID-19 patients with ARDS, odds ratio (OR) 6.2 (95% CI, 1.6–23.4) (5).

In a systematic overview of 80 consecutive autopsies of the COVID-19 deaths, 17 PE (21%) were found, of whom eight were fatal. In each of these deaths as well as in 15 others (32 cases, 40%), lower extremities deep vein thrombosis (LE-DVT) were found. The most frequent cause of death was pneumonia, followed by PE combined with pneumonia (6).

The clinical history and physical examination are of poor utility in determining the probability and risk of deep vein thrombosis (DVT) in the ICU (7, 8). An ultrasound-based DVT screening may be helpful to identify early LE-DVT and therefore adapt treatment in order to prevent progression to PE.

METHOD

Aim of the Study

The aim of the study was to determine the impact of routine screening for DVT on the number of cumulative VTE at day 21 on patients admitted in ICU for COVID-19 pneumonia. The secondary end points were the cumulative incidences of major bleeding and death at day 21.

Study Design and Ethics

This prospective, monocentric, cohort study (ECHO-VID) was conducted in Toulouse University Hospital from March 10, 2020, to May 7, 2020. The management of the patients was not modified during the study, since in the site performing systematic screening for LE-DVT, the ultrasound screening is easily accessible and done, while in the other two centers, it is not. Patients were informed that their data would be used for the study. The local ethic committee gave its consent to the collection of the data and the study is declared in the register of observational studies of Toulouse University Hospital (number's register: 2020-091).

Patients

Consecutive patients admitted in three medico-surgical ICUs for COVID-19 ARDS were identified. SARS-CoV-2 pneumonia was confirmed by a reverse transcription polymerase chain reaction (RT-PCR) test on a nose/throat swab or sputum sample positive for SARS-CoV-2, or, in patients with a negative RT-PCR but with symptoms consistent with COVID-19, by abnormalities highly suspicious of COVID-19 on a chest computed tomography (CT) scan in the absence of an alternative diagnosis. Patients were excluded if their length of ICU stay was <72 h or if acute VTE was already present at ICU admission.

Procedures

The three ICUs follow the same standardized procedures and clinical protocols. Thrombosis prophylaxis was systematically given in COVID-19 patients without major risk of bleeding. Of the three ICUs, one benefitted from the passage of vascular physicians for systematic bedside lower extremities complete compression venous ultrasonography (LE-CCUS screened group) at day 7 ± 2. The other units did not perform systematic screening (non-screened group). All the centers performed CCUS in cases of suspected DVT (localized tenderness, pitting oedema or swelling in each lower extremity, and central venous catheter dysfunction). Proximal DVT was defined as the thrombus involving at least the popliteal vein and above, and distal DVT was defined as the thrombus involving veins below the popliteal level. Isolated DVT was defined as DVT without associated PE. CT pulmonary angiography (CTPA) was performed only for patients with clinical suspected PE (acute degradation of hemodynamic or respiratory status, difficulties to discontinue mechanical ventilation). The three ICUs strictly applied the same thromboprophylaxis protocol: until April 3, a standard dose anticoagulant thromboprophylaxis with enoxaparin 4,000 IU once-daily or 4,000 IU twice-daily for patients with a body mass index >40 kg/m^2. From April 3 onwards, patients received an intermediate-dose anticoagulant thromboprophylaxis with enoxaparin 80 IU/kg per day, in one injection for patients with a body weight of ≤ 100 kg and in two injections for patients with a body weight of >100 kg. In case of severe renal impairment, patients received subcutaneous UFH 5,000 IU two or three times a day without anti-factor Xa monitoring. Patients with acute PE or DVT were treated with therapeutic anticoagulation by intravenous UFH or LMWH (enoxaparin 100 IU/kg twice daily or tinzaparin 175 IU/kg once daily). Patients with indication for oral therapeutic anticoagulation before hospital admission like atrial fibrillation, history of VTE, or mechanical heart valves were switched

Abbreviations: 95% CI, 95% confidence interval; ARDS, Acute respiratory distress syndrome; CCUS, Complete compression venous ultrasonography; COPD, Chronic obstructive pulmonary disease; CTPA, Computed tomography-pulmonary angiography; CT scan, Computed tomography scan; DVT, Deep vein thrombosis; ECMO, Extracorporeal membrane oxygenation; eGFR, Estimated glomerular filtration rate; HR, Hazard ratio; ICU, Intensive care unit; ISTH, International Society of Thrombosis and Haemostasis; LE-DVT, Lower extremity deep vein thrombosis; LMWH, Low-molecular-weight heparin; PE, Pulmonary embolism; RT-PCR, Reverse transcription polymerase chain reaction; SD, Standard deviation; UE-DVT, Upper extremity deep vein thrombosis; UFH, Unfractionated heparin; VTE, Venous thromboembolism.

to enoxaparin 100 IU/kg twice daily, reduced to 100 IU/kg once daily if estimated glomerular filtration rate (eGFR) is between 15 and 29 ml/min/1.73 m^2, or intravenous UFH with a goal of 0.3–0.6 IU/ml of antifactor Xa activity if GFR $\leq$ 14 ml/min/1.73 m^2.

End Points

The primary outcome was the cumulative incidence of objectively confirmed VTE (symptomatic or not), including PE, LE-DVT, and catheter-associated upper extremity (UE)-DVT, during 21 days in the hospital. The secondary outcomes were the cumulative incidences of major bleeding [International Society of Thrombosis and Haemostasis (ISTH)-defined] and death, during the first 21 days following ICU admission (9). We did not adjudicate deaths to identify fatal PE, as all but one death was due to hypoxemic respiratory failure that can be indistinguishable from fatal PE.

Statistical Analysis

Categorical variables are presented as numbers and percentages. Continuous variables are presented as means and standard deviations (SD). Comparisons were made using χ^2 test for categorical variables or Fisher exact test when appropriate, while Student *t*-test and Mann–Whitney test were used for continuous variables. The Kaplan–Meier method was used to estimate cumulative incidence of events, with 95% CI. The association between routine screening using LE-CCUS and VTE diagnosis, major bleeding, and death was analyzed by unadjusted Cox proportional-hazard models, after checking for the proportional-hazard assumption. A *p*-value <0.05 was considered to be statistically significant. Analyses were performed on STATA Statistical software (release 14.2, StataCorp LLC®).

RESULTS

Between March 10 and May 7, 2020, 85 patients were eligible for the study. One patient was excluded due to PE prior to ICU admission, and six patients were excluded because of ICU length of stay <72 h; one of them required extracorporeal membrane oxygenation (ECMO) and underwent a fatal intracranial bleeding 24 h after VTE diagnosis. Finally, 78 patients were included in the analysis. Twenty-seven (34.6%) received systematic screening for DVT by bedside LE-CCUS 7 ± 2 days after ICU admission. The clinical and biological characteristics of the population are presented in **Table 1**. Fifty-five patients underwent CTPA: 22 (81.5%) of the LE-CCUS screened group and 33 (64.7%) of the non-screened group, *p*-value = 0.122.

There were no difference concerning past medical history between the two groups, except for history of transplantation being more frequent in the LE-CCUS screened group (14.8 vs. 2%, *p*-value = 0.046). Of the total population, six patients (7.7%) had a history of VTE, five (6.4%) had atrial fibrillation, two (2.6%) had chronic heart failure, nine (11.5%) suffered from chronic obstructive pulmonary disease (COPD), and six (7.7%) suffered from active neoplasia. Nine (11.5%) were current smokers, 18 (23.1%) had diabetes mellitus, 41 (53.6%) had hypertension, and 17 (21.8%) had dyslipidemia. There was no difference between the two groups regarding ICU organ support therapy, but therapeutic anticoagulation started at ICU admission was more frequent in the LE-CCUS screened group.

During a mean follow-up of 31 ± 20 days, 32 patients (41.0%) were diagnosed with VTE, with a 21-day cumulative incidence of 42.3% (95% CI, 31.4–55.2). There was no difference in VTE cumulative incidence between LE-CCUS screened and non-screened groups (**Figure 1**, **Table 2**). Of note, CCUS was performed in six patients (11.8%) in the non-screened group because of suspected DVT. The type of VTE was PE with or without DVT in 22 patients (28.2%), of whom three were subsegmental PE, proximal LE-DVT in one patient (1.3%), isolated distal LE-DVT in six patients (7.7%), catheter-associated UE-DVT in two patients (2.6%), and catheter-associated UE-DVT + distal LE-DVT in 1 patient (1.3%) (**Table 3**). All but one VTE were diagnosed in patients receiving prophylactic or therapeutic anticoagulation. In the LE-CCUS screened group, the frequency of PE was not reduced (25.9 vs. 29.4%, *p*-value = 0.745), but the frequency of isolated DVT was higher (25.9 vs. 5.9%, *p*-value = 0.027); however, it was mainly isolated distal LE-DVT. After VTE diagnosis, all but one patient received therapeutic anticoagulation, by LMWH or UFH.

There was no difference in the cumulative incidence of major bleeding and death between LE-CCUS screened and non-screened groups (**Table 2**). All but one major bleeding were diagnosed in patients receiving therapeutic or prophylactic anticoagulation, with five ear–nose–throat bleeding, four gastrointestinal bleeding, and two retroperitoneal bleeding. The cause of death was refractory hypoxemia in seven patients (9.0%) and fatal intracranial bleeding in one patient (1.3%) treated by therapeutic dose of UFH for PE.

No statistically significant difference between intermediate-dose and standard dose anticoagulant thromboprophylaxis with enoxaparin was observed for VTE, major bleeding, and death, respective unadjusted HR 0.85 (95% CI, 0.34–2.11), *p*-value = 0.731; 0.66 (95% CI, 0.14–3.09), *p*-value = 0.594; and 0.52 (95% CI, 0.06–4.40), *p*-value = 0.551.

DISCUSSION

Our study confirms the high cumulative incidence of VTE in critically ill patients with COVID-19 pneumonia (42.3%, 95% CI, 31.4–55.2) despite a prophylactic anticoagulation. Previous studies reported that VTE frequency ranged from 6.6 to 37.0% in comparable critically ill patients, despite thromboprophylaxis and without VTE screening, with a 15-day cumulative incidence that ranged from 20.4 to 27% (3, 4, 10–13). The diagnosis was often made within the first week after ICU admission, with a median time from ICU admission of 6 days in the study of Poissy et al. and a median time from hospital admission of 24 h in the study of Lodigiani et al. (4, 12). Frequency of VTE is higher in case of LE-DVT screening by CCUS in ICU, up to 69%, with DVT ranging from 14.7 to 85.4% but mainly distal (14–17). In the only other study evaluating bilateral leg ultrasound screening, the

TABLE 1 | Characteristics of patients in ICU according to systematic screening or not for LE-DVT.

	All patients [*n* = 78 (%)]	LE-CCUS screened group [*n* = 27 (%)]	Non-screened group [*n* = 51 (%)]	*p*-value
Demographic data				
Male sex	67/78 (85.9)	24/27 (88.9)	43/51 (84.3)	0.739
Age (years)	63.3 ± 13.9	61.5 ± 12.4	64.2 ± 14.6	0.411
BMI (kg/m²)	27.7 ± 4.4	28.8 ± 4.0	27.1 ± 4.5	0.107
Days between hospitalization and ICU admission	1.7 ± 2.2	2.0 ± 2.3	1.5 ± 2.2	0.287
SOFA score	5.9 ± 2.2	6.0 ± 2.4	5.9 ± 2.1	0.855
PaO_2/FiO_2 ratio	163 ± 62	167 ± 58	160 ± 64	0.617
Medical history				
History of VTE	6/78 (7.7)	3/27 (11.1)	3/51 (5.9)	0.412
Surgery during the 3 months before	1/78 (1.3)	0/27 (0.0)	1/51 (2.0)	1.000
Confined to bed in hospital during the 3 months before	2/78 (2.6)	0/27 (0.0)	2/51 (3.9)	0.541
Active cancer	6/78 (7.7)	3/27 (11.1)	3/51 (5.9)	0.412
Biologic data at ICU admission				
eGFR (ml/min/1.73 m²)	83 ± 27	82 ± 31	83 ± 24	0.811
Hemoglobin (g/dl)	13.0 ± 2.0	12.6 ± 2.0	13.2 ± 2.0	0.178
Leukocytes (G/L)	7.98 ± 3.44	8.53 ± 3.26	7.68 ± 3.53	0.302
Platelets (G/L)	223 ± 98	229 ± 113	220 ± 91	0.703
D-dimer (mg/L)	1.78 ± 1.07	1.88 ± 1.11	1.69 ± 1.05	0.606
Prothrombin (% of activity)	88 ± 12	86 ± 12	89 ± 12	0.445
aPTT ratio	1.15 ± 0.29	1.22 ± 0.56	1.13 ± 0.11	0.176
Fibrinogen (g/L)	6.8 ± 1.4	7.1 ± 2.0	6.7 ± 1.0	0.353
CRP (mg/L)	137.5 ± 90.0	149.0 ± 93.4	131.8 ± 88.8	0.469
Characteristics of ICU stay				
Prophylactic anticoagulation at ICU admission	69/78 (88.5)	21/27 (77.8)	48/51 (94.1)	0.057
Therapeutic anticoagulation at ICU admission	7/78 (9.0)	5/27 (18.5)	2/51 (3.9)	0.045
Catecholamine support	56/78 (71.8)	20/27 (74.1)	36/51 (70.6)	0.745
Mechanical ventilation	66/78 (84.6)	25/27 (92.6)	41/51 (80.4)	0.200
RRT	5/78 (6.4)	3/27 (11.1)	2/51 (3.9)	0.334
ECMO	5/78 (6.4)	3/24 (11.1)	2/51 (3.9)	0.334
ICU length of stay (days)	21 ± 15	25 ± 16	18 ± 15	0.053
Hospital length of stay since ICU admission (days)	31 ± 20	36 ± 19	29 ± 20	0.117

aPPT, activated partial thromboplastin time; BMI, body mass index; CCUS, complete compression venous ultrasonography; CRP, C-reactive protein; DVT, deep vein thrombosis; ECMO, extracorporeal membrane oxygenation; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; LE, lower extremity; PaO_2/FiO_2 ratio, ratio of arterial oxygen partial pressure to fractional inspired oxygen; RRT, renal replacement therapy; SOFA, sequential organ failure assessment. Results are expressed as numbers and percentages [n (%)] or mean ± standard deviation.

21-day cumulative incidences of VTE were 59% (95% CI, 42–72) with a screening approach for LE-DVT and 34% (95% CI, 21–46) when excluding asymptomatic events detected by LE-CCUS (18). Isolated distal DVT are frequent and represent 30–50% of all LE-DVT diagnosed on CCUS series (19–21). Therapeutic anticoagulation is not mandatory if the risk of recurrence is low (22).

To date, routine ultrasound screening for the detection of asymptomatic LE-DVT in COVID-19 patients is not recommended (23, 24). However, to the best of our knowledge, our study is the first to compare LE-CCUS screened and non-screened groups, with no difference found regarding cumulative incidences of VTE, major bleeding, and death. Because the majority of PE originated in the deep venous system of LE, undiagnosed DVT and resultant PE may be an important contributor to hypoxic pulmonary vasoconstriction that would lead to pulmonary hypertension and right ventricular failure in COVID-19 patients, in addition to worsening of ARDS (25). However, in contrast with the relatively frequent report of PE in hospitalized COVID-19 patients, LE-DVT, especially proximal, might be less common and some authors hypothesize that the observed pulmonary vessel occlusions are caused by local thrombi in pulmonary arteries, as a consequence of vascular damage associated with viral infection and severe inflammation, rather than emboli from peripheral veins. LE-DVT screening to prevent PE in this setting might be ineffective (26, 27).

Our study has some limitations. First, the sample size is relatively small due to low peak of COVID-19 patients in our ICU during the study period. Therefore,

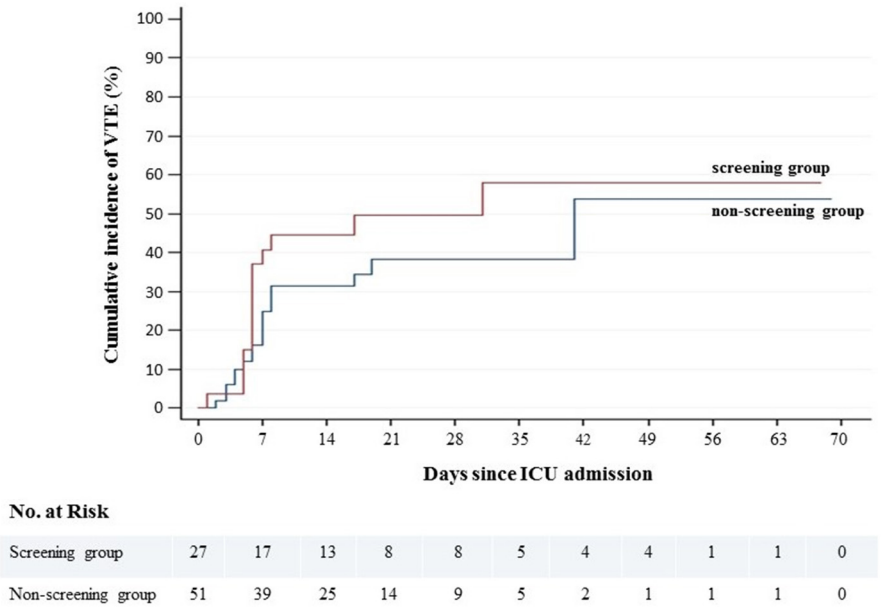

FIGURE 1 | Cumulative incidence of venous thromboembolism according to screening or not for LE-DVT.

TABLE 2 | Cumulative incidence of events at 21 days according to systematic screening or not for LE-DVT, and comparison by unadjusted Cox models, with non-screened group as reference.

	All [% (95% CI)]	LE-CCUS screened group [% (95% CI)]	Non-screened group [% (95% CI)]	Hazard ratio (95% CI)	*p*-value
Venous thromboembolism	42.3 (31.4–55.2)	49.5 (32.2–69.9)	38.3 (25.3–55.1)	1.45 (0.72–2.93)	0.296
Major bleeding	9.6 (4.7–19.2)	14.8 (5.8–34.8)	6.9 (2.3–20.0)	1.05 (0.36–3.09)	0.924
Death	10.3 (5.0–20.8)	3.7 (0.5–23.5)	14.0 (6.4–29.2)	0.26 (0.03–2.10)	0.206

CCUS, complete compression venous ultrasonography; DVT, deep vein thrombosis; LE, lower extremity.

TABLE 3 | Clinical outcomes of patients according to systematic screening or not for LE-DVT.

	All [*n* = 78 (%)]	LE-CCUS screened group [*n* = 27 (%)]	Non-screened group [*n* = 51 (%)]	*p*-value
Venous thromboembolism	32/78 (41.0)	14/27 (51.9)	18/51 (35.3)	0.157
Pulmonary embolism	22/78 (28.2)	7/27 (25.9)	15/51 (29.4)	0.745
Associated with LE-DVT	9/78 (11.5)	7/27 (25.9)	2/51 (3.9)	0.007
Without LE-DVT	13/78 (16.7)	0/27 (0.0)	13/51 (25.5)	0.003
Isolated DVT	10/78 (12.8)	7/27 (25.9)	3/51 (5.9)	0.027
Deep vein thrombosis localization				
Proximal LE-DVT	3/78 (3.9)	3/27 (11.1)	0/51 (0.0)	0.038
Isolated distal LE-DVT	14/78 (18.0)	11/27 (40.7)	3/51 (5.9)	<0.001
Catheter-related UE-DVT	3/78 (3.9)	1/27 (3.7)	2/51 (3.9)	1.000
Major bleeding	14/78 (18.0)	6/27 (22.2)	8/51 (15.7)	0.541
Death	8/78 (10.3)	1/27 (3.7)	7/51 (13.7)	0.250

CCUS, complete compression venous ultrasonography; DVT, deep vein thrombosis; LE, lower extremity; UE, upper extremity. Results are expressed as numbers and percentages [n (%)] or mean ± standard deviation.

this study has not enough power to detect a difference in mortality between groups. Secondly, LE-DVT screening was based on a single LE-CCUS at 7 ± 2 days. In literature, ultrasound screening protocol vary from one single examination (time from ICU admission not specified) to repeated examinations every 5–7 days (14–16, 18). The

results could have been different with earlier screening or repeated LE-CCUS.

In conclusion, a systematic approach with screening for DVT at 7 ± 2 days of ICU admission does not appear to be associated with a higher diagnosis of VTE or a lower cumulative incidence of PE. Further studies are needed to evaluate the value of routine screening for DVT in critically ill patients admitted for COVID-19 pneumonia.

DATA AVAILABILITY STATEMENT

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Toulouse University Hospital local ethic committee (number's register: 2020-091). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

F-XL and FV-B designed the study, performed data collection and analysis, interpreted the data, wrote the manuscript, and gave final approval of the manuscript before submission. VM and AB-R designed the study, interpreted the data, revised the manuscript, and gave final approval of the manuscript before submission. AR interpreted the data, wrote the manuscript, and gave final approval of the manuscript before submission. BC and AT performed data collection, interpreted the data, revised the manuscript, and gave final approval of the manuscript before submission. TG and SS interpreted the data, revised the manuscript, and gave final approval of the manuscript before submission. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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ACE Gene I/D Polymorphism and Acute Pulmonary Embolism in COVID19 Pneumonia: A Potential Predisposing Role

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Most recent studies have stressed a high risk of thromboembolism in patients with SARS-CoV-2 infection, particularly in those with severe COVID-19 pneumonia. Counterbalance between angiotensin-converting-enzyme (ACE) and ACE2 activities in COVID-19 disease may be crucially involved in the thrombo-inflammatory process. Currently, no study has investigated ACE I/D polymorphism involvement in COVID-19 disease complicated by pulmonary embolism, hence the aim of the present pilot study. This is a retrospective, single-center observational case-control study, conducted at the Sub-Intensive Care Unit of A.O.R.N. Ospedali dei Colli, Cotugno Hospital, Naples (Italy). We included 68 subjects with severe/critical COVID-19 pneumonia. COVID-19 patients were divided according to occurrence of PE (PE+, $n = 25$) or absence of thromboembolic complications (PE−, $n = 43$). Assessment of ACE I/D polymorphisms showed a statistically significant difference between PE+ and PE− patients ($p = 0.029$). Particularly, prevalence of D/D homozygous polymorphism was significantly higher in PE+ COVID-19 patients than in PE− (72 vs. 46.5%; $p = 0.048$), while heterozygote I/D polymorphism was significantly lower expressed in PE+ patients than in PE− (16 vs. 48.8%; $p = 0.009$). Computed tomographic pulmonary angiography showed predominantly mono/bilateral sub-segmental embolisms. In conclusion, our findings let us hypothesize a genetic susceptibility to thromboembolism in COVID-19 disease. ACE D/D polymorphism might represent a genetic risk factor, although studies on larger populations are needed.

Keywords: COVID19 pneumonia, pulmonary embolism, ace gene, polymorphism, angiotensin II

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Calabrese C, Annunziata A, Coppola A, Pafundi PC, Guarino S, Di Spirito V, Maddaloni V, Pepe N and Fiorentino G (2021) ACE Gene I/D Polymorphism and Acute Pulmonary Embolism in COVID19 Pneumonia: A Potential Predisposing Role. Front. Med. 7:631148. doi: 10.3389/fmed.2020.631148

INTRODUCTION

Most recent studies have stressed a high risk of thromboembolism in patients affected by SARS-CoV-2 infection, particularly in those with severe COVID-19 pneumonia.

Laboratory findings from retrospective cohort studies suggest an activation of the coagulation cascade characterized by elevated levels of D-dimer and fibrinogen in association with other altered

coagulation indexes (1, 2). In addition, anticoagulant therapy with low molecular weight heparin reduced mortality rates of patients affected by severe SARS-CoV2 pneumonia showing an increase either in D-dimer levels and/or high sepsis-induced coagulopathy score (3, 4).

Venous thromboembolism in COVID-19 patients has been largely reported most in Intensive Care Units, with a prevalence ranging from 17 to 69% (5–9).

Nonetheless, the pathogenesis of the increased thromboembolic risk in COVID-19 pneumonia still remains unclear. Several mechanisms have been hypothesized as involved in this process, i.e., the direct cytotoxic effect induced by the virus, the endothelial cell inflammation and the dysregulated immune response, which ultimately result in the recruitment of inflammatory cells, platelet aggregation and activation of the complement and coagulation cascade (10).

In addition, the counterbalance between angiotensin-converting enzyme (ACE) and ACE2 activities occurring in COVID-19 may play a crucial role in the thrombo-inflammatory process. ACE cleaves Angiotensin (Ang) I to produce Ang II, whilst ACE 2 converts Ang II in the protective Ang 1-7. The loss of ACE2, the receptor of the SARS-CoV2 spike protein, leaves unopposed effects of Angiotensin II, thus leading to vasoconstriction, endothelial injury, endovascular thrombosis, and increased blood volume (10, 11).

In the literature, several polymorphisms of ACE gene have been described, among which the either presence (insertion, allele I) or absence (deletion, allele D) of a 287-base pair (bp) Alu repeat sequence in intron 16. A strong association between these polymorphisms and serum levels of ACE has been reported, with D/D homozygotes having 65% more, and I/D heterozygotes 31% more ACE than I/I homozygotes (12).

Indeed, several studies have also demonstrated an association between the frequency of ACE D/D polymorphism and both the prevalence and the mortality rates of COVID-19 (13, 14).

Up to now, no study has investigated the involvement of ACE I/D polymorphism in COVID-19 complicated by pulmonary embolism, hence the aim of the present pilot study.

MATERIALS AND METHODS

Study Design

This is a retrospective, single-center observational case-control study, conducted at the Sub-Intensive Care Unit of A.O.R.N. Ospedali dei Colli, Cotugno Hospital, Naples (Italy). We included all patients suffering from COVID-19 pneumonia hospitalized between March 20, 2020 and July 20, 2020 with suspect of pulmonary embolism.

Severe/Critical COVID-19 patients, according to the World Health Organization classification (15), were divided into two subgroups according to their either occurrence or not of pulmonary embolism (PE) during SARS-CoV2 lung infection. The diagnosis of pulmonary embolism was performed by computed tomographic pulmonary angiography and lower-limb compression ultrasonography.

We compared our COVID-19 ACE genotype frequencies with those from the Italian general population reported on https://alfred.med.yale.edu/alfred/index.asp.

All subjects provided their written informed consent to the treatment of their data for clinical and research purposes. The study was approved by the local Ethic committee of AORN Ospedali dei Colli and it is in accordance with the 1976 Declaration of Helsinki and its later amendments.

Parameters

Upon admission, all patients were asked for collection of anamnestic and anthropometric data (age, sex, BMI, smoking habit), presence of comorbidities (systemic arterial hypertension, type 2 diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, deep venous thrombosis, and obesity) and medication intake (e.g., antihypertensives such as ACE inhibitors or AT1 receptor antagonists).

All patients underwent to molecular analysis for thrombophilia and cardiovascular risk factors, performed by our Hospital Central Laboratory using a Reverse Dot Blot (RDB) kit by Nuclear Laser Medicine (NLM, version 2020.10.19, CVD-14 cod. AC084, Milan). In depth, we focused on Angiotensin I Converting Enzyme (ACE I/D polymorphism) (rs1799752 SNP): I/I = Insertion in homozygosis, I/D = Insertion/Deletion, and D/D = Deletion in homozygosis.

Statistical Analysis

Categorical variables were expressed as number and percentage whilst continuous variables by median and interquartile range, after Shapiro-Wilk test to assess for normal distribution. The differences between groups were evaluated either by the Fisher's exact test or Chi-square test, depending on the sample size. Where appropriate, Yates correction was applied to the Chi Square test (in case of cell width <5 and table "m X n") and Dunn-Sidak correction to Fisher Exact test. For all statistical tests, we used the Stata 15.0 (StataCorp, Texas, USA) and SPSS 24.0 (SPSS INC, Chicago, IL, USA). Statistical significance was defined as $p < 0.05$ and all p-values were two-tailed.

RESULTS

General Characteristics of the Study Population

The overall study population included 68 patients with either severe or critical COVID-19 pneumonia.

COVID-19 patients were further divided into two subgroups according to the occurrence, during hospitalization, of PE (PE+, $n = 25$) or absence of any thromboembolic complication (PE−, $n = 43$). The median age of the study population was 58.5 years [IQR: 46.3–66], and they were mainly males (69.1%). As for smoking, the 50% were non-smokers, only the 2.9% were smokers, whilst the 39.7% were past-smokers.

The most prevalent comorbidity was arterial hypertension (50%), while diabetes was present in 14.7% of patients, cardiovascular disease in the 11.8% and COPD in 7.4%.

In depth, among the 34 patients with arterial hypertension, 8 (23.5%) were on ACE inhibitors and 11 (32%) on angiotensin receptor blockers.

The comparison of demographic, clinical and laboratory characteristics between the two subgroups (PE+ vs. PE−) did not shown any statistically significant difference, except for a higher prevalence of smoking habit and an increase in serum C-reactive protein in the PE+ group.

General characteristics of the study population are described in **Table 1**.

Assessment of ACE I/D Polymorphism

Table 2 describes the allelic and genotypic frequencies of the rs1799752 SNP. We compared the genetic frequencies of the rs1799752 SNP of PE+ and PE− COVID-19 patients with the Italian control population (CP) published by the Yale Genom Databank website (https://alfred.med.yale.edu/alfred/index.asp). From Alfred database we know that 222 ACE I/D alleles were studied, with an I allele frequency of 34.2% (76 alleles) vs. 65.8% for D allele (146 alleles). So there are 13 I/I; 50 I/D; and 48 D/D in the control population. Thus, checking for Hardy-Weinberg equilibrium (HWE): PE+ p-value is 0.027, so HWE is not satisfied. Conversely, PE− p-value is 0.456 so the two populations are in HWE.

As for allele differences between the three subgroups (controls, PE+ and PE− COVID-19 patients) no significant difference was found at Fisher's test ($p = 0.500$), whilst there was a significant difference between groups as for genotypes ($p = 0.029$).

Looking at differences among genotypes between the three subgroups, the following results were obtained. Comparison between CP and PE+ genotypes (13 50 48; 3 4 18) showed a p-value = 0.013, whilst CP vs. PE− (13 50 48; 2 21 20) a p-value = 0.447 and, finally, PE+ vs. PE− (3 4 18; 2 21 20) a p-value = 0.015. Sorting into ascending and using Dunn-Sidak thresholds order we found a difference between CP and PE+ (0.013 < 0.017) and between PE+ and PE− (0.015 <

TABLE 1 | Baseline clinical and laboratory characteristics of the overall study population and study subgroups according to the presence of pulmonary embolism (PE+ and PE−) ($n = 68$).

Parameter	Overall Population ($n = 68$)	PE + ($n = 25$)	PE – ($n = 43$)	*P*
Age (yrs.), median [IQR]	58.5 [46.3-66]	62 [49–67.5]	57 [42–65]	0.315
Sex, *n* (%)				0.502
M/F	47 (69.1)/21 (30.9)	19 (76)/6 (24)	29 (68.3)/14 (31.7)	
BMI (kg/m2), median [IQR]	28 [26-30.3]	28 [27–31.5]	27 [24.5–29.2]	0.182
Obesity, *n* (%)	20 (29.4)	8 (32)	12 (27.9)	0.781
Smoking habit, *n* (%)				**0.035**
Yes	2 (2.9)	2 (8)	-	
No	34 (50)	17 (68)	22 (48.8)	
Ex	27 (39.7)	6 (24)	21 (51.2)	
Hypertension, *n* (%)	34 (50)	13 (52)	21 (48.8)	0.897
Diabetes, *n* (%)	10 (14.7)	3 (12)	7 (16.3)	0.859
CAD, *n* (%)	8 (11.8)	-	8 (18.6)	0.051
COPD, *n* (%)	5 (7.4)	1 (4)	4 (9.3)	0.711
Deep Venous Thrombosis, *n* (%)	1 (1.5)	1 (4)	-	0.801
Pulmonary Embolism, *n* (%)	25 (36.8)			n.a.
Laboratory				
CRP (mg/dL), median [IQR]	11.7 [3.4–20.3]	13.7 [11–20.5]	3.5 [0.4–13.9]	**0.014**
IL-2R (IU), median [IQR]	913 [500–1561]	932 [764–1428]	614 [275–1565.5]	0.369
IL-6 (pg/mL), median [IQR]	57 [20.7–207]	59 [25–211]	52.5 [6.6–174.9]	0.367
PT, median [IQR]	84 [71–92]	80 [66–102]	91 [75–92]	0.397
aPTT, median [IQR]	34.1 [31.7–36.6]	33.9 [30.6–36]	34.2 [32.9–43.1]	0.456
PT-INR, median [IQR]	1.17 [0.93–1.27]	1.18 [1.08–1.44]	1.06 [0.8–1.22]	0.295
D-dimer (ng/mL), median [IQR]	497 [172–3189]	2235 [246–5959]	204 [163–1186]	0.222
Fibrinoge *n* (mg/dL), median [IQR]	534 [380–756]	576 [359–842]	427. [371–734]	0.628
P/F, median [IQR]	145 [102–285]	125 [98–200]	182 [122–322]	0.121
Therapy				
Ace-inhibitors, *n* (%)	8 (11.8)	4 (16)	4 (9.3)	0.610
Sartans, *n* (%)	11 (16.2)	4 (16)	7 (16.3)	1.000

PE, pulmonary embolism; M, male; F, female; BMI, Body Mass Index; CAD, Coronary Artery Disease; COPD, Chronic Obstructive Pulmonary Disease; CRP, C reactive protein; IL, interleukin; PT, prothrombin time; P/F: PaO2/FiO2; IQR, interquartile range.

Reference ranges: IL-2R: 223–710; IL-6: 0–5; PT: 70–125; aPTT: 25–40; PT-INR: 0.8–1.2; D-dimer: <250 ng/mL; Fibrinogen: 175–417.

TABLE 2 | Allelic and genotypic frequencies and Hardy Weinberg Equilibrium test for ACE I/D polymorphism rs1799752 in PE+ and PE− COVID-19 patients, and in the control population.

Parameter	Control Population ($n = 222$)	COVID-19 PE+ ($n = 25$)	COVID-19 PE− ($n = 43$)	p
Allele, *n* (%)				0.500
I	76 (34.2)	7 (24.1)	23 (35.9)	
D	146 (65.8)	22 (75.9)	41 (64.1)	
Genotype, *n* (%)				**0.029**
I/I	13 (11.7)	3 (12)	2 (4.7)	
I/D	50 (45)	4 (16)	21 (48.8)	
D/D	48 (43.3)	18 (72)	20 (46.5)	
HWE		0.027	0.456	

**Values are expressed as absolute numbers (%). p-values were estimated with Fisher Exact Test with Dunn-Sidak correction.*
***HWE: Hardy Weinberg Equilibrium; PE: Pulmonary Embolism; ACE: Angiotensin I Converting Enzyme.*

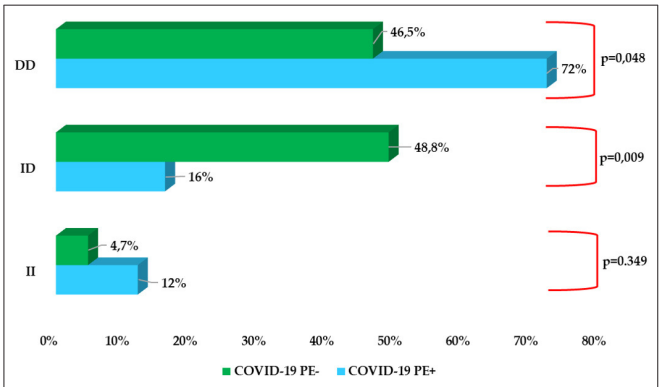

FIGURE 1 | Prevalence of ACE I/D polymorphism rs1799752 in COVID-19 PE+ subgroup vs COVID-19 PE− subgroup.

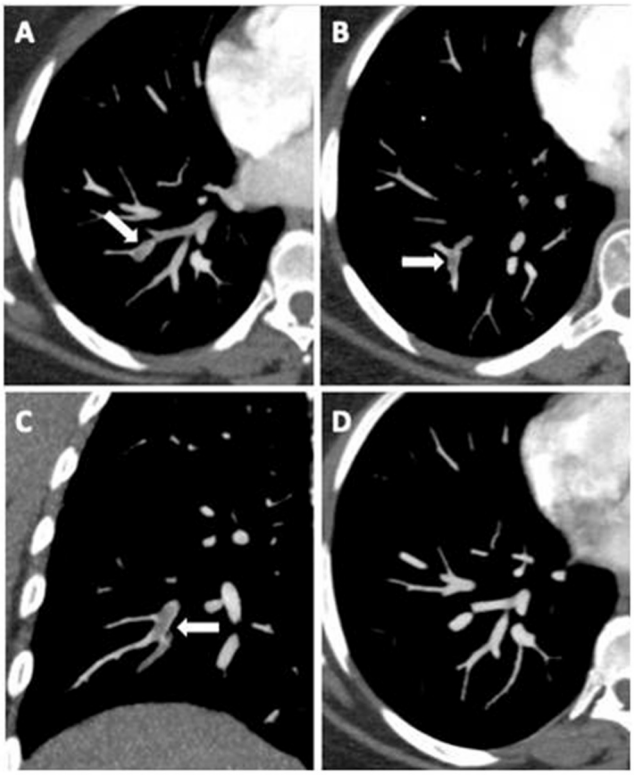

FIGURE 2 | Axial CT images **(A,B)** and coronal multiplanar reconstruction **(C)** document partial centric and eccentric opacification defects in the right lower lobe basal segmental arteries (white arrows), suggestive of acute pulmonary embolism. Complete regression after anticoagulant therapy **(D)**.

0.025), whilst no difference emerged as between CP and PE− (0.447 > 0.050).

A significantly higher prevalence of D/D homozygous polymorphism in PE+ COVID-19 patients than in PE− (72% vs. 46.5%; $p = 0.048$) was observed, while heterozygote I/Dpolymorp hism presented a significantly lower expression in PE+ patients rather than in PE− (16% vs. 48.8%; $p = 0.009$). No significant difference instead emerged with regard to homozygote polymorphism I/I ($p = 0.349$). Computed tomographic pulmonary angiography showed predominantly mono- and bilateral sub-segmental embolisms (**Figure 2** and **Supplementary Figure 1**).

DISCUSSION

In the present study we observed, for the first time, a significant higher prevalence of D/D ACE polymorphism in severe COVID-19 patients who developed pulmonary embolism as compared to those without thromboembolic complications. As compared with the general population, indeed, we observed a statistically significant difference as compared to COVID-19 PE+ patients, whilst no difference emerged as for comparison with COVID-19 PE− subgroup.

A recent study reported a higher prevalence of ACE D/D genotype in severe COVID-19 patients as compared to those with mild-disease, even though this association was dependent on the presence of hypertension comorbidity (16). ACE D/D polymorphism has been associated not only with hypertension, though also with obesity and diabetes, chronic conditions highly suggestive of high risk for COVID-19 infection, as well as for poor outcomes of the disease (17).

In addition, demographic studies have found in the racial variance of ACE I/D genotype a potential explanation of the different prevalence and outcomes due to COVID-19. In fact, the higher frequency of the D allele seems to perfectly match with the higher mortality rates observed in the African American population, as compared to Indians and White people, and in the European populations (particularly Italian, Spanish, and French) as compared to the Asian ethnic group (13, 14).

Likewise, a recent study reported an inverse correlation between ACE I/I genotype and both the prevalence and the mortality due to SARS-CoV-2 infection (18). Moreover, a meta-analysis demonstrated an association between the I/D allele

frequency ratio and the recovery rate, though not for mortality rates (19). On the contrary, a study on people from Europe, North Africa and the Middle East demonstrated an inverse correlation between prevalence and mortality due to COVID-19 and the ACE D allele frequency (20).

In patients affected by acute respiratory distress syndrome (ARDS), previous studies have demonstrated an association between the D/D genotype and incidence, morbidity and mortality risk (21–24). In addition, SARS patients with an ACE D/D genotype disclosed a more severe degree of the disease, with the frequency of D allele higher in the hypoxemic group (25).

ACE D/D genotype has been suggested as a susceptibility marker of thrombosis. In fact, ACE D/D homozygosis has been associated to thromboembolism occurrence in subjects actually without predisposing factors and traditional thrombophilic alterations in other disease (26).

In the present study we did not find any significant difference of serum D-Dimer levels between COVID 19 patients either with or without pulmonary embolism. This finding could be related to the small number of patients recruited in the study. In contrast, the increase in C-reactive protein observed in patients undergoing to pulmonary embolism suggests the crucial role of inflammation in the pathogenesis of thrombotic complications in patients affected by SARS-CoV 2 infection.

In conclusion, the results of the present study let us hypothesize a genetic susceptibility to thromboembolism occurring in COVID 19 disease. ACE D/D polymorphism linked to higher levels of both ACE and angiotensin II could represent a genetic risk factor, although studies recruiting larger cohorts of patients are needed.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: available after evaluation of the specific request and need. Requests to access these datasets should be directed to cecilia.calabrese@unicampania.it.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethic Committee of AORN Ospedali dei Colli and University of Campania Luigi Vanvitelli. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study design and conception was made by CC and AA. CC and GF were responsible for the whole content of the study and contributed to the draft of the manuscript. AC and VD managed data collection. PP was responsible for the data handling, statistical analysis, and data interpretation. SG performed and analyzed tomographic pulmonary angiography. All authors have read, written and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.631148/full#supplementary-material

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D-Dimer-Driven Anticoagulation Reduces Mortality in Intubated COVID-19 Patients: A Cohort Study With a Propensity-Matched Analysis

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Objective: Examine the possible beneficial effects of early, D-dimer driven anticoagulation in preventing thrombotic complications and improving the overall outcomes of COVID-19 intubated patients.

Methods: To address COVID-19 hypercoagulability, we developed a clinical protocol to escalate anticoagulation based on serum D-dimer levels. We retrospectively reviewed all our first 240 intubated patients with COVID-19. Of the 240, 195 were stratified into patients treated based on this protocol (ON-protocol, $n = 91$) and the control group, patients who received standard thromboprophylaxis (OFF-protocol, $n = 104$). All patients were admitted to the Stony Brook University Hospital intensive care units (ICUs) between February 7th, 2020 and May 17, 2020 and were otherwise treated in the same manner for all aspects of COVID-19 disease.

Results: We found that the overall mortality was significantly lower ON-protocol compared to OFF-protocol (27.47 vs. 58.66%, $P < 0.001$). Average maximum D-dimer levels were significantly lower in the ON-protocol group (7,553 vs. 12,343 ng/mL), as was serum creatinine (2.2 vs. 2.8 mg/dL). Patients with poorly controlled D-dimer levels had higher rates of kidney dysfunction and mortality. Transfusion requirements and serious bleeding events were similar between groups. To address any possible between-group differences, we performed a propensity-matched analysis of 124 of the subjects (62 matched pairs, ON-protocol and OFF-protocol), which showed similar findings (31 vs. 57% overall mortality in the ON-protocol and OFF-protocol group, respectively).

Conclusions: D-dimer-driven anticoagulation appears to be safe in patients with COVID-19 infection and is associated with improved survival.

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What This Paper Adds: It has been shown that hypercoagulability in patients with severe COVID-19 infection leads to thromboembolic complications and organ dysfunction. Anticoagulation has been variably administered to these patients, but it is unknown whether routine or escalated thromboprophylaxis provides a survival benefit. Our data shows that escalated D-dimer driven anticoagulation is associated with improved organ function and overall survival in intubated COVID-19 ICU patients at our institution. Importantly, we found that timely escalation of this anticoagulation is critical in preventing organ dysfunction and mortality in patients with severe COVID-19 infection.

Keywords: D-dimer-driven anticoagulation, anticoagulation, d-dimer, thrombotic complications, hypercoagulability, COVID-19

INTRODUCTION

Thrombosis is a major cause of morbidity and mortality in severe COVID-19 illness. Deep vein thrombosis (DVT) (1), pulmonary embolism (PE) (2, 3), cerebral infarction (4), and myocardial infarction (MI) (5, 6) have all been reported in patients severely ill because of COVID-19 (7–9). Additionally, vascular access including dialysis catheters (10) and extracorporeal membrane oxygenation (ECMO) circuits (11) also fail at higher rates. D-dimer levels, a marker of fibrin breakdown (12) and intravascular clot burden (13), are increased in many patients with COVID-19. The degree of elevation correlates with disease severity (14) and it is particularly high in patients who died (15). Furthermore, widespread microthrombosis in the kidneys (16) and lungs (17), and other organs has been reported at autopsy. These observations strongly indicate that coagulopathy in COVID-19 infection contributes to organ dysfunction and mortality.

Anticoagulation appears to improve outcomes in critically ill COVID-19 patients, according to an early report from China (18), as well as a larger series from New York (19). However, two reports from France reported unexpected PE despite therapeutic anticoagulation (20, 21), presumably due to the severity of the hypercoagulability. Recently, a group in Italy reported that non-critically ill patients did not benefit from elevated doses of anticoagulation, though details were lacking about what agents and doses were administered (22). Additionally, an Italian group reported improved mortality in a retrospective cohort that received an "intermediate" dose of low molecular weight heparin (LMWH, 40–60 mg twice daily) (23). These findings were echoed in a report by five centers in New York City in which both prophylactic and therapeutic anticoagulation were associated with improved mortality, although there was not a significant difference between these groups ($p = 0.07$) (24). Despite these promising signs, a large randomized trial of therapeutic anticoagulation (NIH ACTIV-4) recently paused enrollment for lack of efficacy in critically-ill patients (25). At present, no randomized data is available to support one approach over another.

Despite the dearth of data, several professional societies have promulgated guidelines on anticoagulation in COVID-19. The American Society of Hematology (ASH) currently recommends against the use of therapeutic doses of heparin or LMWH in these patients in the absence of confirmed or suspected VTE (26). However, the International Society of Thrombosis and Hemostasis (ISTH) also recommends routine thromboprophylaxis with subcutaneous unfractionated heparin (UFH) or LMWH, with consideration for intermediate doses of LMWH in high-risk patients. The guidelines also state to consider a 50% increase in the dose of thromboprophylaxis in obese patients (27). Finally, current NIH treatment guidelines also indicate that there is not enough data available to recommend the use of anticoagulants at higher doses in these patients (28). Thus, new data are urgently needed.

During the early phase of the pandemic, our group observed severe arterial and venous thrombosis in COVID-19 patients despite routine, standard low-dose thromboprophylaxis. Based on these early observations, we hypothesized that escalated thromboprophylaxis for hypercoagulability (as indicated by elevated D-dimer levels) would limit thrombotic complications and improve outcomes in COVID-19 infection. We also suspected that many patients with increasing D-dimer levels had occult thromboses, either PE, or clots in other vascular beds. Therefore, we developed a protocol to escalate the level of anticoagulation, based on serum D-dimer levels, measured on a daily basis.

In the current report, we reviewed all of our COVID-positive, intubated patients (n = 240) admitted between February 7th, 2020 and May 17, 2020. We describe the 91 intubated COVID-19 intensive care unit (ICU) patients who received thromboprophylaxis based on this protocol, during the first wave of the pandemic in New York. At the peak of the pandemic, two of five ICUs at our institution had agreed on the escalated anticoagulation protocol; the 91 reported patients were admitted to these ICUs, based on pure chance availability of beds. This random assignment (a sort of "experiment of nature") gives us the opportunity to understand whether escalated anticoagulation had a benefit, vs. routine care. We compared these patients to a cohort of 104 ICU patients who either received routine thromboprophylaxis or started full dose anticoagulation when standard clinical indications (e.g. DVT, PE) became apparent. Both groups were admitted to the hospital and ICU during the same time period, and their clinical care was otherwise similar.

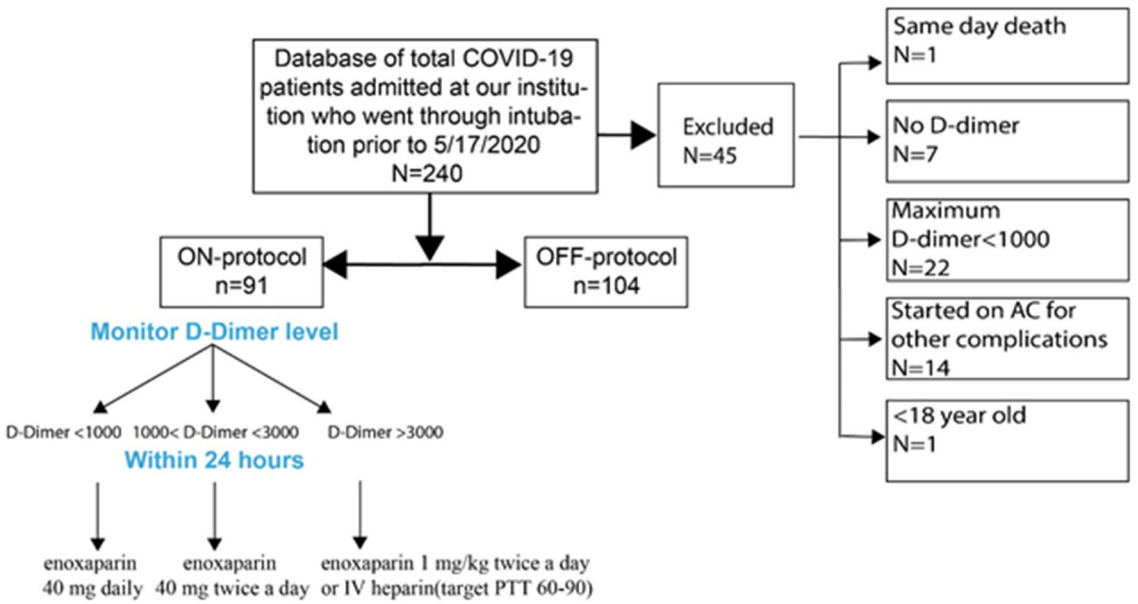

FIGURE 1 | Patient selection algorithm and the anticoagulation protocol.

METHODS

Ethics Statement

This study was a retrospective chart review of a COVID-19 patient database. Stony Brook University Committee on Research in Human Subjects approved the study protocol and supervised all study procedures, in accordance with state and federal regulations, with a waiver of informed consent.

Target Population and Data Sources

We identified all intubated COVID-19 patients admitted to Stony Brook University Hospital between February 7, 2020 and May 17, 2020 with a positive RT-PCR test for SARS-CoV-2 (**Figure 1**). Our initial screen identified 240 intubated patients. We then applied our inclusion/exclusion criteria:

Inclusion Criteria

1. Age $\geq$ 18
2. COVID-19 infection with a positive RT-PCR test
3. Respiratory failure requiring endotracheal intubation
4. At least two D-dimer measurements after intubation
5. D-dimer elevation to >1,000 ng/mL during ICU course.

Exclusion Criteria

1. Oral anticoagulation prior to and on admission
2. Therapeutic anticoagulation initiated because of cardiac arrhythmia
3. Pregnancy or delivery within 2 weeks of intubation
4. Death in the Emergency Department
5. Known bleeding diathesis or hypercoagulability
6. D-dimer levels < 1,000 ng/mL throughout hospitalization
7. No D-dimer levels sent during hospitalization.

Patients were then stratified to ON- and OFF-protocol. ON-protocol patients met the following criteria:

1. Anticoagulation administration was based on D-dimer level, in the following sliding scale:

   a. D-dimer < 1,000 ng/mL: enoxaparin 40 mg daily
   b. D-dimer $\geq$ 1,000 ng/mL but < 3000 ng/mL: enoxaparin 40 mg twice a day
   c. D-dimer $\geq$ 3,000 ng/mL: enoxaparin 1 mg/kg twice a day, or therapeutic anticoagulation with IV heparin (target PTT 60-90), based on physician preference.

2. Escalation of anticoagulation occurred within 24 h of a change in the D-dimer level.

OFF-protocol patients met the inclusion criteria but did not meet ON-protocol requirements. In general, physicians adhered to the protocol, with some bias toward administration of anticoagulation at lower D-dimer levels than prescribed (**Supplementary Figure 1**). In many cases, enoxaparin was used despite creatinine elevation; there was no obvious increase in hemorrhagic complications (see **Table 1**). In many OFF-protocol patients, anticoagulation was administered eventually, for other clinical reasons or more than 24 h after changes in D-dimer. No patients in this group were initially administered anticoagulation at ICU admission, and we excluded patients given heparin or other anticoagulation because of cardiac arrhythmia from this study. Additionally, patients who did not meet inclusion criteria are excluded from this report. Adjudication of patients to the two groups was done by three authors and classification was by consensus.

TABLE 1 | Cause of death.

	Cause of death					
Groups	**MOF**	**Suspected PE**	**Hypoxic respiratory failure**	**MI**	**CNS complications**	**Aspiration**
ON-protocol	14	4	5	1	1	0
OFF-protocol	42	13	2	1	2	1
TOTAL	56	17	7	2	3	1

Random Assignment to ICUs

The chance circumstance that made the comparison between ON- and OFF-protocol groups possible was adoption of escalated anticoagulation in two of five ICUs at our institution. In the remaining three ICUs, anticoagulation was administered in routine fashion (see Results). Assignment to different ICUs was a random process, based on bed availability.

Chart Review

We reviewed each chart and collected the following data:

1. Demographics
2. Dates of admission and intubation
3. Comorbidities
4. Laboratory data
5. Adverse events from COVID-19 (death, thromboembolic phenomena, renal failure). We documented "suspected PE" in patients who were previously on stable ventilatory settings and suddenly developed acute respiratory deterioration with increased needs for ventilatory support and concomitant circulatory collapse, not able to be attributed to other causes (sepsis, myocardial infarction, pneumothorax, mucous plug, etc.). Due to the extreme precautions to control the dissemination of the SARS-CoV-2 virus along with patients' hemodynamic instability, we decided to treat those patients preemptively as having PE, without any confirmatory imaging test.
6. SOFA score—this score was calculated with lab values sent at the time of intubation and for 24 h subsequently (29). If a lab value was not available immediately, it was carried forward from admission labs. Phenylephrine was converted to norepinephrine equivalents as suggested by Lambden (30).
7. Clinically significant bleeding defined as:
    1. Gastrointestinal bleeding requiring transfusion of at least two units of red blood cells (RBCs);
    2. Hemoglobin < 7 mg/dL and transfusion of at least two units of RBCs;
    3. Intracranial bleeding or
    4. Other major bleeding requires transfusion including massive hemoptysis, hematuria, retroperitoneal hematoma, intraperitoneal, or intrathoracic bleeding.
8. Long-term outcomes (death, discharge from hospital) are reported if available. For all 195 patients, 4 months of follow-up data were available. To this date, 96.4% (188/195) of patients have either been discharged from the hospital or deceased. All patients were included in the Kaplan-Meier analysis.

Data Analysis

Laboratory Analysis

We generated time series data in MATLAB representing D-dimer levels and other laboratory values, time-locked to three main dates: admission date, intubation date, and anticoagulation starting date. We collected laboratory values for all patients (except for one patient with missing laboratory data) and calculated the mean and standard error (SE) for both groups. The ACL TOP Hemosil D-dimer HS (high sensitivity) test was used to assess D-dimer levels.

Statistics

Statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, Ill) and in-house developed coding in MATLAB. The significance level for all tests was 0.05. All reported P values were calculated two-sided. The primary endpoint was death. Secondary endpoints included discharge.

Data were reported as group means, along with the two-tailed Student's T-statistic for several labs (D-dimer, BUN, creatinine). We had hypothesized that these specific values would be different and thus no multiple comparisons correction is appropriate. Non-parametric analysis was performed to compare the means of maximum D-dimer, creatinine, BUN, and SOFA score. Other categorical variables such as hypertension, chronic kidney disease, chronic obstructive pulmonary disease (COPD), sex, and diabetes were compared using the $\chi 2$ test. Two-sample T-test or Mann-Whitney U-tests were used for continuous variables as indicated based on normal distribution vs. skewness of factors.

Survival and its association with measured factors were evaluated using Kaplan-Meier models. A log-rank test was used to compare survival between groups. There was no missing data regarding survival measures. We used Cox proportional-hazards regression models to estimate the predictors of survival. The multivariable Cox regression model included participation in the protocol, gender, age, SOFA score, and BMI. Entry-level for multivariable analysis was $P < 0.1$. The multivariable model had an excellent fit with $P < 0.001$. Hazard ratios were calculated to estimate independent predictors of survival.

Propensity-Score Matched Analysis

We performed a propensity score-matched analysis of 122 of the subjects to isolate the effect of anticoagulation on outcome. We used logistic regression to calculate a propensity score (31) and matched cases using the "Greedy algorithm" (32). Regression

TABLE 2 | Patient demographics and adverse events.

	OFF-Protocol (*N* = 104)	ON-Protocol (*N* = 91)	*P* value
Age—year (mean ± SE)	61.7 ± 1.6	57.7 ± 1.6	0.079
Male (%)	76 (73.07)	65 (71.14)	0.79
SOFA score (mean ± SE)	6.97 ± 0.24	6.19 ± 0.21	0.21
Max D-dimer (mean ± SE)	12343 ± 1318	7553 ± 972	0.005
Admission Creatinine (mean ± SE)	1.41 ± 0.15	1.114 ± 0.08	0.1
BMI (mean ± SE)	30.04 ± 0.63	30.27 ± 0.6	0.79
Intubation (%)	104 (100)	91 (100)	
Death (%)	61 (58.6)	25 (27.47)	<0.001
Discharged (%)	39 (37.5)	62 (68.13)	<0.001
Days from intubation to death	17 ± 1.63	19 ± 2.52	
PE(including suspected PE) /DVT (%)	24 (23.07)	9 (9.8)	<0.014
Received Transfusion (%)	49 (47.11)	45 (49.4)	0.74
COMORBIDITIES			
HTN (%)	58 (55.7)	46 (50.5)	0.46
COPD (%)	5 (4.8)	6 (6.5)	0.58
Heart failure	6 (5.7)	2 (2.1)	0.2
Diabetes	29 (27.88)	30 (32.9)	0.44
CKD (%)	7 (6.7)	3 (3.2)	0.27

Group characteristics and adverse events. Groups were similar at baseline. The ON-protocol group had significantly lower D-dimer levels, fewer deaths, and fewer pulmonary emboli and deep vein thromboses. Categorical values were compared with chi-square statistics. Independent-samples Mann-Whitney U tests were used for noncategorical variables which could not be assumed to be distributed normally.

model variables included age, gender, BMI, SOFA score, heart disease, diabetes, and hypertension, and excluded pairs with the distance of PS score > 0.01. Additional variables were excluded because of relatively low numbers.

RESULTS

Study Population

After initial screening, from a total of 240 patients admitted to Stony Brook University Hospital ICUs between February 7, 2020, and May 17, 2020, 195 patients were included for analysis. The exclusion criteria can be found in **Figure 1**. Patients were randomly assigned to one of five ICUs on admission, based on bed availability. During the first wave of COVID-19 cases at our institution, there was a wide inter-practitioner variation in thromboprophylaxis. However, physicians in two of our five ICUs rapidly agreed on a protocol for escalated anticoagulation, based on D-dimer levels, because of the clinical observation of severe thromboembolic events (see Methods). Hospital leadership promulgated official guidelines for the care of COVID-19 patients on March 25th, leading to the relative uniformity of care; nearly all patients included in the study were intubated after this date (**Figure 3A**). We stratified the 195 patients into ON-protocol ($n = 91$) and OFF-protocol ($n = 104$) groups. Note that both groups were admitted to the hospital and ICU during the same time period. The mean ages in each group were similar (57.7 vs. 61.7, $P = 0.079$), as were other demographic features, and antiviral drugs and steroids were used at similar rates (**Table 2**, **Supplementary Table 1**). Most patients were given hydroxychloroquine and steroids; rates were similar in ON-protocol and OFF-protocol groups. However, very few patients received remdesivir (13%, 18.6% ON-protocol, and 8.6% OFF-protocol). However, remdesivir did not change the mortality in this subgroup (**Supplementary Figure 2**, left panel). Exclusion of patients who were treated with remdesivir did not change the study overall results (**Supplementary Figure 2**, right panel). We calculated initial SOFA scores for each patient upon intubation. The distribution of SOFA scores is shown in **Supplementary Figure 3** (ON protocol: 6.19; OFF protocol, 6.97, Mann-Whitney *U*-test, $P = 0.21$). We also accounted for this difference with a propensity-matched analysis (see below).

ON-Protocol Patients had Low Mortality

Overall cumulative mortality (with a minimum of 4 months of follow up for all the patients) for ICU patients with severe COVID-19 was 44% (86/195, **Figure 2A**, **Table 2**). Kaplan-Meier survival analysis demonstrated that ON-protocol group patients had significantly lower mortality rates compared to the OFF-protocol group (**Figure 2B**, overall mortality 27.47 vs. 58.6%, $P < 0.001$; **Table 2**).

In univariate survival analysis, patients in the OFF-protocol group ($P < 0.0001$), male patients ($P = 0.051$) with age greater/equal to 70 years ($P < 0.001$), and SOFA score greater/equal to seven ($P < 0.001$) were each associated with lower rates of survival. The multivariable analysis shows that OFF-protocol group membership was an independent predictor of higher mortality (hazard ratio [HR], 2.33; 95% confidence interval [CI], 1.4-3.75; $P = 0.0001$). In the multivariable analysis of mortality, male sex (HR, 1.79; 95% CI, 1.04-3.07; $P = 0.034$), SOFA score greater/equal to seven (HR, 2.16; 95% CI, 1.33-3.5; $P = 0.002$), and age over 70 (HR, 2.02; 95% CI, 1.28-3.17; $P = 0.002$) were also predictors of poor outcome. Importantly, both groups were admitted to the hospital and ICU during the same time period (**Figure 3A**). Cumulative mortality increased rapidly in the OFF-protocol group, while discharges were more common in the ON-protocol group (**Figure 3B**).

Propensity-Matched Analysis

To account for possible differences between the study groups, we performed a propensity score-matched analysis. We were able to match 124 patients within a propensity score of <0.01. Patients who received ON-protocol anticoagulation had a mortality of 31 vs. 57% mortality in the OFF-protocol matched cohort (**Figure 4**). Importantly, Kaplan-Meier curves of ON-protocol and OFF-protocol groups for the propensity-matched groups were similar to the Kaplan-Meier curve obtained from the whole sample.

Anticoagulation Per-Protocol Robustly Controls D-Dimer Levels and Kidney Function

In addition to lower mortality, the ON-protocol group displayed a higher hospital discharge rate compared to the OFF-protocol group (**Figure 3B**). To uncover the potential mechanism underlying this fast recovery and improved outcome, we analyzed

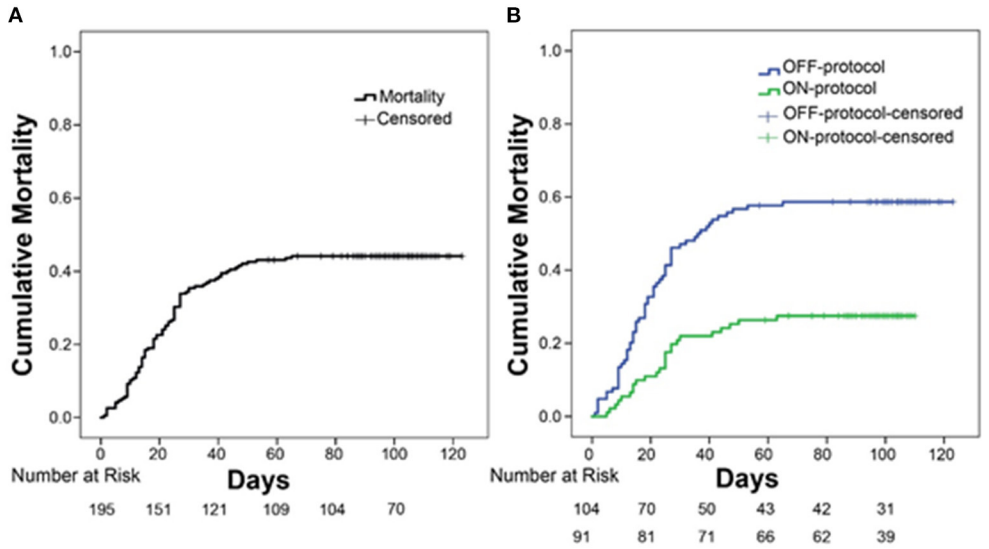

FIGURE 2 | Protocol-driven anticoagulation is associated with significantly lower mortality. **(A)** Overall mortality in intubated patients with COVID-19 infection who were admitted to the ICU. **(B)** Comparison of overall mortality between ON-protocol group (green line, $N = 91$) and OFF-protocol group (blue line, $N = 104$) (log-rank test, $P < 0.001$).

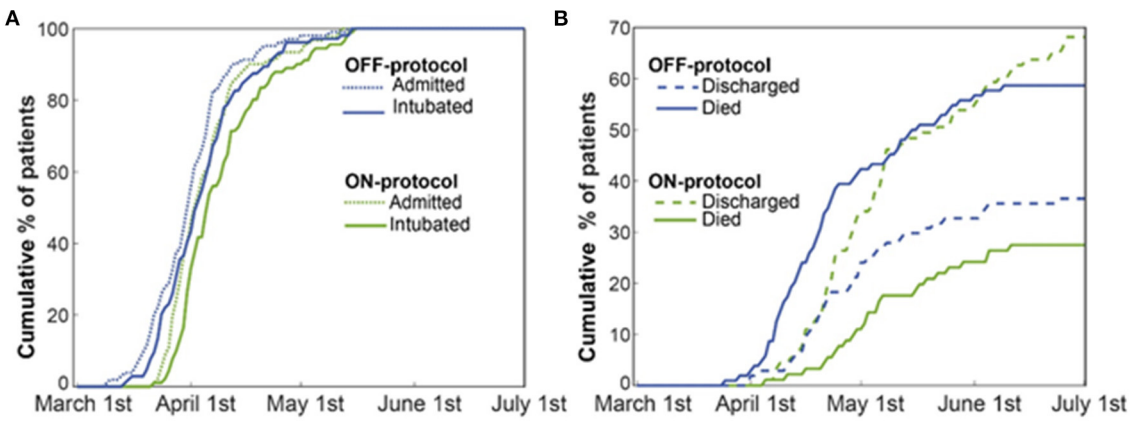

FIGURE 3 | The ON-protocol and OFF-protocol groups were admitted at similar time periods but with drastically different outcomes in terms of mortality and discharged rates. **(A)** The admission and intubation timeline of both ON (green) and OFF-protocol (blue) groups are shown. **(B)** For both the ON- (green lines) and OFF-protocol (blue lines) groups, we plotted the accumulated percentages of the discharged and expired patients. Dashed and solid lines, respectively, represent the accumulated percentages of discharged and expired patients in each group. The overall mortality rate is 58.65% in the OFF-protocol group compared to the 27.47% in the ON-protocol group, while patients in the ON-protocol group were discharged at a much higher rate (69.23% compared to 37.5% in the OFF-protocol group).

serial D-dimer levels in patients who were ON- and OFF-protocol, as well as creatinine and BUN values over time. **Figure 5** shows how these laboratory values (mean ± SE), time-locked to the intubation date, develop over days after intubation. These results revealed that early anticoagulation robustly controlled D-dimer levels. During the first two weeks after intubation, D-dimer, creatinine, and BUN were especially elevated in OFF-protocol patients. This is a critical time period associated with most of the deaths in COVID-19 intubated patients, and most of the mortality in the OFF-protocol patients occurred during this time period (associated with the steep slope of the mortality curve in **Figure 3B**). The mean maximum D-dimer level was 7,553 ng/mL (median 4028 ng/mL) for ON-protocol patients, and 12,343 ng/mL (median 7030 ng/mL) for OFF-protocol (Mann-Whitney U-test, $P = 0.001$, **Figure 5,** left panel). Patients who were ON-protocol also had lower creatinine levels (mean of maximum value for ON-protocol = 2.2 mg/dL, median = 1.23 mg/dL, SE = 0.22, and OFF-protocol = 2.81 mg/dL, median = 1.98 mg/dL, SE = 0.24, Mann-Whitney U-test, $P < 0.019$, **Figure 5**, middle panel). In contrast, BUN (ON-protocol = 66.23 mg/dL [median = 47 mg/dL], SE = 4.68, OFF-protocol = 77.63 mg/dL (median = 68.3 mg/dL), SE = 5.07, $P < 0.126$) did not achieve statistically different values in the aggregate, although the trend was different (**Figure 5**). Anticoagulation,

therefore, was associated with superior kidney function and overall outcome.

Adverse Events

The ON-protocol group experienced a lower incidence of thromboembolic complications. Four patients who expired had suspected PE. Two patients were diagnosed with segmental PEs on imaging, and three patients were found to have DVTs. One patient was diagnosed with cerebral infarction after extubation. Six patients had arterial thromboses: four patients who were diagnosed with non-ST elevation myocardial infarction (NSTEMI), one with splenic infarcts and one with lower extremity arterial embolism.

By contrast, in the OFF-protocol group, 13 patients who expired had suspected PE. Five surviving patients had imaging-confirmed PE and six were diagnosed with DVT (total of 23% PE/DVT compared to 9.8% in ON-protocol patients, $p = 0.014$). Eleven patients had arterial thromboses: two patients suffered from ischemic stroke, seven patients had clinically significant MI, one acute limb ischemia and one mesenteric ischemia.

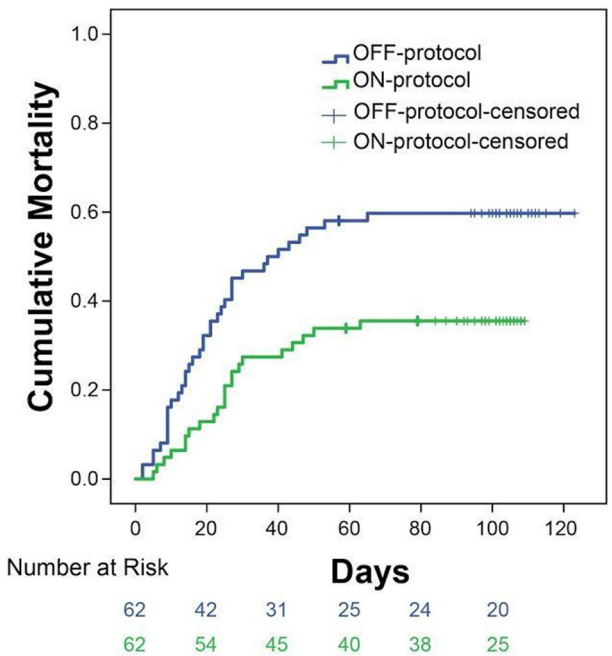

FIGURE 4 | Subgroup analysis of 124 patients matched from ON-protocol ($n = 62$) and OFF-protocol groups ($n = 62$) exhibits similar results as the whole group analysis. Sixty two pairs of ON and OFF-protocol patients were analyzed on age, gender, BMI, SOFA score, heart disease, diabetes, and hypertension with the distance of a PS score ≤ 0.01. The other comorbidity variables were not used because the values were ≤ 10. The mortality rates in these groups are 31% (ON-protocol) vs. 57% (OFF-protocol, $P = 0.0061$).

Bleeding Complications Were Similar Between Groups

Bleeding complications were frequent but similar between groups. Nine patients in the ON-protocol group developed upper or lower GI bleeding, manifesting as melena, blood in the orogastric tube or hematochezia, and four more required transfusions due to bloody respiratory secretions, hemothorax, mediastinal, and tracheostomy site bleeding. A total of 19 patients experienced a hemoglobin drop to <7 mg/dL at some point during hospitalization and one patient had a hemorrhagic stroke.

In the OFF-protocol group, nine patients developed GI bleeding presenting in the same way as the ON-protocol patients, and nine required transfusions for retroperitoneal bleeding, hematuria, hemothorax, and bloody respiratory secretions. A total of 21 patients developed a hemoglobin <7 mg/dL and two experienced intracranial bleeding.

PRBC transfusion unit requirements were very similar between two groups (ON-protocol, median 0, range 0-18, mean 2.38, SE 0.39; OFF-protocol, median 0, range 0–24, mean 2.9, SE 0.48; Mann Whitney U-test, $P = 0.989$).

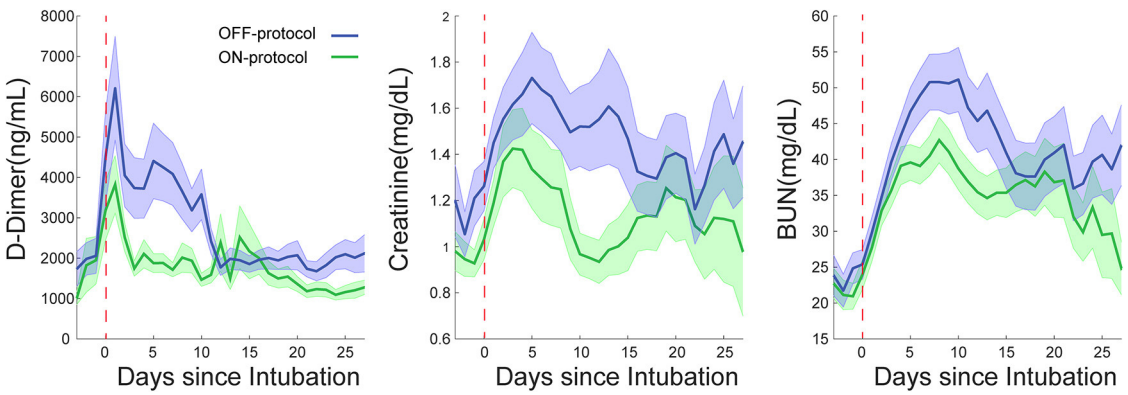

FIGURE 5 | Escalated, D-dimer driven anticoagulation (ON-protocol group) is associated with improved critical laboratory values in multiorgan dysfunctions in COVID-19 intubated patients. The early start of AC in the ON-protocol group was associated with significant changes in the course of the disease in intubated patients. The green and blue lines represent the mean of D-dimer, creatinine and BUN for ON- and OFF-protocol groups over thirty days. Notice the elevated level of these laboratory values in the first two weeks following intubation, which is associated with many of mortalities in the OFF-protocol group. The shaded area represents the SE of the mean. These analyses are time-locked to the intubation date marked by the red dashed line.

DISCUSSION

Our study results indicate that an early-onset escalating thromboprophylaxis protocol based on daily D-dimer level is associated with significantly fewer thrombotic complications, preserved kidney function, and improved mortality in intubated patients with severe COVID-19 infection. The mortality in the ON-protocol cohort was 27%, compared to 58% in the OFF-protocol group, although this comparison needs to be made with caution, given the fact that this study is not a randomized trial, and it is possible there were unobserved differences between the groups that account for the differences in mortality. Nonetheless, the propensity-matched analysis also supports our core hypothesis, which should be confirmed in larger, randomized trials. The outcomes in the ON-protocol group are superior to those described in other published reports (33) and is probably due to both prevention of large-vessel thrombosis and improvement of kidney function, possibly by prevention of microthrombosis. Many fewer patient deaths in the ON-protocol group were attributed to large arterial or venous thrombotic complications, and clinically significant hemorrhage was not different between groups. On the contrary, thirteen patients in the OFF-protocol group died during their ICU course from probable PE, despite being administered standard low-dose thromboprophylaxis.

Our findings are generally consistent with the reported high incidence of thrombotic complications in COVID-19 ICU patients (7–9). At present, there is no official guidance about anticoagulation in COVID-19, except for previously promulgated guidelines (26–28, 34). There are reports indicating some benefit of anticoagulation particularly in critically ill patients with COVID-19 infection (18, 19), but the anticoagulation type is variable, and timing of onset is not reported, so comparisons are difficult. Of note, Paranjpe et al. (19) reported a similar mortality benefit for anticoagulation in intubated patients (29 vs. 62% mortality), though no further details about these subgroups are available in their manuscript. The report of Nadkarni and colleagues which did not identify a significant difference between therapeutic and prophylactic anticoagulation made no effort to propensity match or establish that the groups receiving prophylactic and/or therapeutic anticoagulation were similar. Thus, their data should not be over-interpreted to claim there is no benefit to therapeutic anticoagulation. By contrast, our data, while not conclusive, support the view that escalated anticoagulation may be appropriate when the D-dimer level rises. We designed our protocol to escalate the intensity of anticoagulation based on D-dimer levels because of the reported association of higher D-dimer levels to increased mortality (15, 35). We believed that early thromboprophylaxis would control the prothrombotic effect of severe COVID-19 infection, prevent early death from thrombotic complications, and limit the extent of microthrombi, thus preventing patient progression to multi-system organ failure (MSOF). This notion is supported by our analysis which indicates that ON-protocol anticoagulation controls the D-dimer level, prevents the occurrence of thromboembolic complications, preserves organ perfusion (as measured by preserved renal function), and is the only independent predictor of patient survival, and was accomplished without an increased risk of the need for transfusion. Our data underscore the importance of the timing of the anticoagulation. This early D-dimer driven escalation could also explain why the ON-protocol mortality we observed is lower than what has been reported in the literature for intubated ICU patients, whether they received anticoagulation or not (15, 33).

LIMITATIONS

This study has several limitations, inherent to the single-institution, retrospective design with a small sample size and the fact that it is subject to residual confounding. Since the two groups of patients were treated in different ICUs, we cannot eliminate with certainty the possibility that other aspects of patient's care might explain the difference in the outcome. When these patients were becoming critically ill, our institution was in the rapidly escalating pandemic curve. In this phase bed availability and the patient assignment were a random event. COVID-19 treatment protocols have been otherwise consistent in our hospital for critically ill patients throughout the pandemic. The anticoagulation protocol was addressed and implemented institution-wide later allowing for this difference in care. However, we did not observe any major differences in the management other than the protocol for anticoagulation, and the propensity-matched analysis was similar. Nevertheless, propensity-matched analysis has its limitations, including the fact that in developing the propensity scores, important variables that could have affected the outcome may have been inadvertently omitted. Moreover, only two-thirds of the patients were able to be matched. Thus, while the use of anticoagulation was associated with improved outcomes, causality cannot be proved. Furthermore, creatinine was slightly more elevated in the OFF-protocol group, although the mean SOFA scores at protocol initiation were not significantly different. We do think comparisons between the two groups should be made with caution, but the outcomes of the OFF-protocol group are similar to those described in the literature (15, 19, 33). Additionally, our study did not include any comparison with patients not receiving anticoagulation, and we did not do further analysis to compare the two types of anticoagulation regimens that were used; low-molecular-weight heparin and unfractionated heparin. Our cohort was relatively overweight (mean BMI of 30); it is possible that a thinner cohort would have fewer thromboembolic complications. However, obesity is now a well-established risk factor for severe COVID-19 disease (36–38), and at least one report describes the mean BMI in their cohort as 29 (39). Thus our cohort's BMI is probably fairly representative of other critically-ill patients. Finally, although the differences in outcomes in the groups studied herein are impressive, the fact that pulmonary embolism/thrombosis symptoms frequently overlap those of severe COVID-19 infection, and that imaging was underutilized to prevent unnecessary staff exposure, might

have led to underdiagnosis of thromboembolic complications. This fact might explain the higher D-dimer levels and higher mortality rate that was observed in the OFF-protocol group. And while recent commentaries call for controlled trials of anticoagulation in patients with COVID-19 (40), we believe the dramatic difference in outcomes revealed by these data should be carefully considered in designing and awaiting results of a double-blinded, controlled trial. These findings and the success of this protocol that has the longest follow-up among all published studies, provide a window toward understanding the mechanisms driving excessive thrombosis and its treatment in this disease.

CONCLUSION

Protocol-driven anticoagulation was safe and effective in the treatment of a cohort of COVID-19 patients and associated with significantly lower mortality and improved kidney function. Our findings should be validated in a larger randomized, controlled trial.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the authors upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Stony Brook University Committee on Research in Human Subjects. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AT, SM, CM, NL, MB, DR, JR, PD, PV, JV, MT, and KK: study conception and design. AT, JR, MB, DR, MT, and JV: designed the clinical protocol. SM, WH, and MB: data analysis and making figures. AT, SM, CM, NL, MB, DR, JR, PD, PV, NC, LA, AS, AO, WH, JV, MT, and KK: planning the data analysis and data interpretation. PD, PV, NC, LA, AS, AO, SM, JR, CM, and AA: data acquisition. AT, CM, SM, NL, MT, and KK: drafting the manuscript. All authors: critically revising the manuscripts. All the authors gave the final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.631335/full#supplementary-material

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COVID-19 and Risk of Acute Ischemic Stroke and Acute Lung Injury in Patients With Type II Diabetes Mellitus: The Anti-inflammatory Role of Metformin

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Background: Coronavirus disease 19 (COVID-19) is regarded as an independent risk factor for acute ischemic stroke (AIS) due to the induction of endothelial dysfunction, coagulopathy, cytokine storm, and plaque instability.

Method: In this retrospective cohort study, a total of 42 COVID-19 patients with type 2 diabetes mellitus (T2DM) who presented with AIS within 1 week of displaying COVID-19 symptoms were recruited. According to the current anti-DM pharmacotherapy, patients were divided into two groups: a Metformin group of T2DM patients with COVID-19 and AIS on metformin therapy (850 mg, 3 times daily ($n = 22$), and a Non-metformin group of T2DM patients with COVID-19 and AIS under another anti-DM pharmacotherapy like glibenclamide and pioglitazone ($n = 20$). Anthropometric, biochemical, and radiological data were evaluated.

Results: Ferritin serum level was lower in metformin-treated patients compared to non-metformin treated patients (365.93 ± 17.41 vs. 475.92 ± 22.78 ng/mL, $p = 0.0001$). CRP, LDH, and D-dimer serum levels were also lowered in metformin-treated patients compared to non-metformin treated patients ($p = 0.0001$). In addition, lung CT scan scores of COVID-19 patients was 30.62 ± 10.64 for metformin and 36.31 ± 5.03 for non-metformin treated patients.

Conclusion: Metformin therapy in T2DM patients was linked to a lower risk of AIS during COVID-19. Further studies are needed to observe the link between AIS in COVID-19 diabetic patients and metformin therapy.

Keywords: COVID-19, acute ischaemic stroke, diabates mellitus, metformin, SARS-CoV-2

INTRODUCTION

Coronavirus disease 19 (COVID-19) is a worldwide pandemic, caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), that began in December 2019. Among the multiple mechanisms of virus action, the ability of the spike protein to bind to angiotensin converting enzyme 2 (ACE2) is the most prominent one, being around 10 times higher than the equivalent SARS-CoV (1). The ACE2 receptors involved in viral entry are highly expressed in different tissues, mainly in lung pneumocyte type II cells. The interaction between SARS-CoV-2 and ACE2 leads to a down-regulation of the protective ACE2 with the induction of hyper-inflammation and oxidative stress, and subsequent development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (2). Also, a reduction of ACE2, which is involved in the metabolism of angiotensin II (AngII), leads to vasoconstriction, hypertension, coagulopathy, and inflammatory reactions that together increase the risk of acute ischemic stroke (AIS) (3). Belani et al. (4) found that COVID-19 is regarded as an independent risk factor for AIS due to the induction of endothelial dysfunction, coagulopathy, cytokine storm, and plaque instability. A systemic review and meta-summary by Tan et al. (5) illustrated that high levels of D-dimer and inflammatory cytokines with the existence of anti-phospholipid antibodies seem to be linked to AIS in COVID-19 patients. On the other hand, the metabolic disturbances associated with type II diabetes mellitus (T2DM) may raise the risk of AIS during SARS-CoV-2 infection. This is because both T2DM and COVID-19 are linked to platelet activation, coagulation disorders, endothelial dysfunction, and insulin resistance (IR) that mutually contribute to the pathogenesis of AIS (6, 7). Also, Lee et al. (8) confirmed that glucose variability is associated with stroke severity and infarct volume in T2DM and non-DM patients. COVID-19 progression is accompanied with glucose variability due to the induction of IR and/or pancreatic injury by hypercytokinemia (9).

Metformin is a biguanide anti-diabetic agent used as a first-line drug in T2DM management, with anti-inflammatory and antioxidant properties (10). As its main actions, metformin increases ACE2 expression, thereby reducing the deleterious effect of high AngII in patients with cardiometabolic disorders and in the experimental model of ALI (11). A preliminary prospective study by Gao et al. (12) found that metformin therapy in COVID-19 patients with T2DM led to a raise in COVID-19 severity through potentiation of SARS-CoV-2 entry due to ACE2 receptors' overexpression. Likewise, ACE2 receptors improve neuronal functions and have neuroprotective activity, being down-regulated in AIS (13).

As a consequence, the rational of the present study was supported by the fact that the anti-inflammatory and antioxidant effects of metformin may improve the cardiometabolic profile in T2DM patients and COVID-19 (14). Thus, this study was aimed to illustrate the potential and bidirectional effect of metformin on both AIS and ALI in T2DM patients with COVID-19.

MATERIALS AND METHODS

Study Design

In this retrospective cohort study, a total of 42 COVID-19 patients with T2DM with ages ranging from 41 to 66 years (12 females and 30 males) who presented with AIS within 1 week of showing COVID-19 symptoms were recruited from a single institutional COVID-19 sector and compared to 21 matched healthy controls. All COVID-19 patients with AIS were diagnosed cooperatively by an internist and neurologist using a full medical record, physical and neurological examinations, as well as biochemical and serological investigations. According to the current anti-diabetic pharmacotherapy, the patients were divided into two groups: Group I (the Metformin group), comprising T2DM patients with COVID-19 and AIS on metformin therapy (850 mg, 3 times daily) ($n = 22$), and Group II (the Non-metformin group), comprising T2DM patients with COVID-19 and AIS on another anti-diabetic pharmacotherapy ($n = 20$).

AIS patients were selected according to the diagnostic criteria of the American Academy of Neurology (15). The diagnosis of COVID-19 was done according to the COVID-19 diagnostic criteria reported by Ma et al. (16). All study procedures were done according to the Helsinki Declaration.

Recruited patients and healthy controls gave informed consent for their contribution to this study. This study was done in the Department of Clinical Pharmacology and Therapeutic and was approved by the Clinical Research and Ethical Committee Board, College of Medicine, Al-Mustansiryia University, Iraq, Bagdad, reference number MTR 21/3/2020.

Inclusion and Exclusion Criteria

In this study, inclusion and exclusion criteria were defined as follows. T2DM patients aged > 40 years who presented with COVID-19 and AIS with/without metformin therapy were included in this study. History of cigarette smoking was considered in the inclusion criteria. On the other hand, patients with hemorrhagic stroke, T1DM, thyroid disorders, ischemic heart disease, valvular heart diseases, acute and chronic liver disorders, acute and chronic renal disorders, bacterial sepsis, mental and psychiatric disorders, complicated stroke, connective tissue disorders, or malignancies were excluded.

Radiological Imaging

Brain computed tomography (CT) scan was done to confirm the focal neurological injury within 48 h from admission of AIS patients. Also, chest X-ray and lung CT scan investigations were done to confirm the existence of bilateral ground glass appearance in suspected COVID-19 patients. Lung CT scan scoring was done according to Francone et al. (17), where a score of 0: no lung involvement; score 1: < 5% involvement of lung; score 2: involvement of 5–25% of lung; score 3: involvement of 26–50% of lung; score 4: involvement of 51–75% of lung; and score 5: involvement >75% of lung.

Anthropometric Measurements

Body mass index (BMI) was measured by the specific equation: BMI = Weight (kg)/Height (m^2). Systolic and diastolic blood

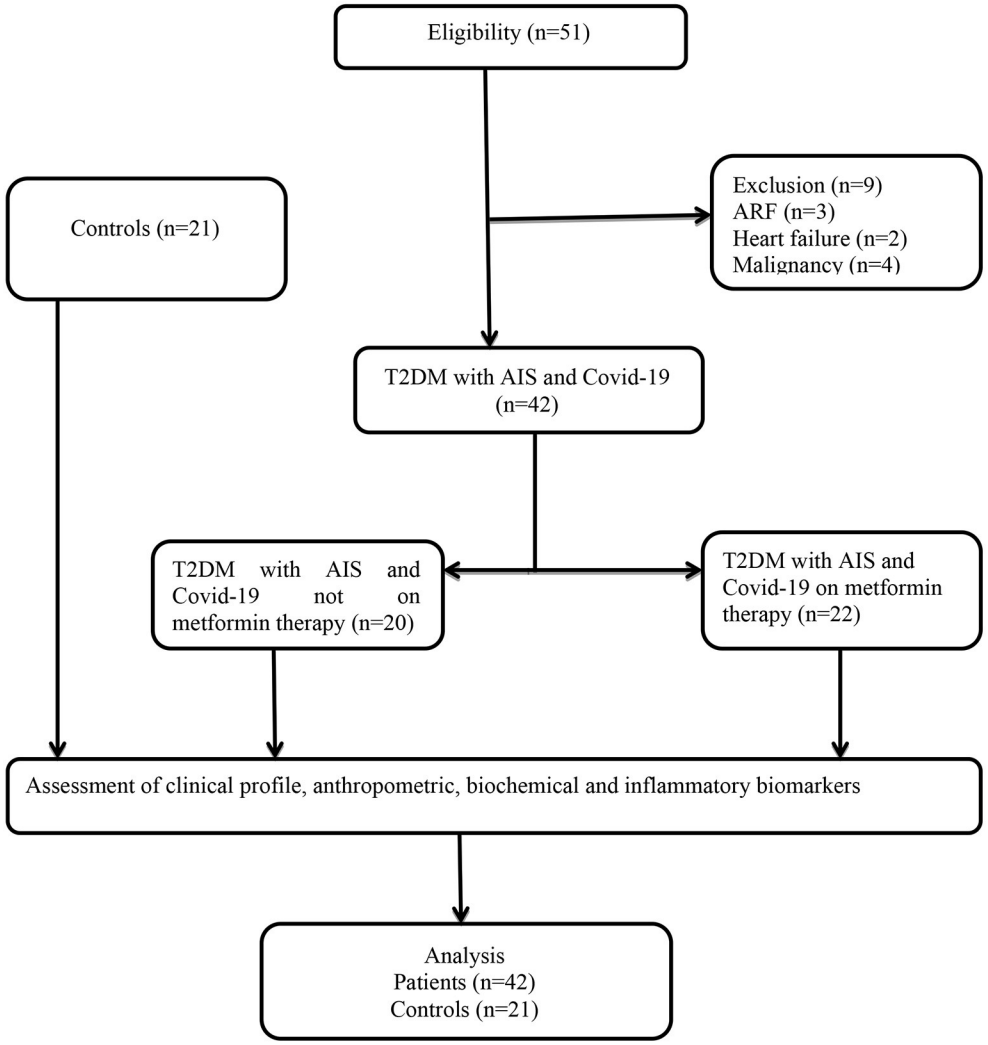

FIGURE 1 | Consort-flow chart of the study.

pressure (SBP and DBP) were measured from the left arm at a supine position using an automated digital sphygmomanometer. Pulse pressure (PP) and mean arterial pressure (MAP) were estimated according to the Al-Kuraishy et al. (14) method.

Biochemical Measurements

Following overnight fasting, 10 ml of venous blood sample was drawn for the assessment of inflammatory biomarkers and glycemic indices. Fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) were measured by automated colorimetric assay. A homeostatic model for the assessment of insulin resistance (HOMA-IR) was used for determination of insulin resistance (IR). Lipid profile, including total triglyceride (TG), total cholesterol (TC) and high-density lipoprotein (HDL), were measured by the ELISA kit method. Other lipid profiles were measured indirectly by specific equations, including low density lipoprotein (LDL) by Friedewald formula, atherogenic index (AI) = log (TG/HDL), very-low-density lipoprotein (VLDL) = TG/5, atherogenic coefficient (AC) = TC-HDL/HDL, and cardiac risk ratio (CRR) = TC/HDL (18).

Serum ferritin (NV: 20–250 ng/ml), C-reactive protein (CRP) (NV: 0–5 mg/L), lactate dehydrogenase (LDH) (NV: 100–190 U/L), D-dimer (NV: < 230 ng/ml), and serum insulin levels were measured by ELISA kit methods. Each sample was measured twice and a mean of the values was used to minimize measurement errors.

Serological Investigations

A polymerase chain reaction anti-COVID-19 test to detect both immunoglobulin (Ig) M and IgG antibodies was used. The cut-off value was 0.00–0.04 mIu/ml for both IgM and IgG.

Estimation of Stroke Risk Score

Stroke risk score (SRS) was estimated in AIS patients and healthy controls according to sheet questionnaires that considered age,

TABLE 1 | Patients' demographic characteristics.

Parameters	Healthy controls	Covid-19/AIS/T2DM patients	*p* value
n	21 (33.30)	42 (66.67)	0.01
Age (years)	48.08 ± 6.51	47.28 ± 6.73	0.65
Male gender	14 (66.67)	30 (71.42)	0.69
Positive family history	3 (14.28)	29 (69.04)	0.001
Smoking	5 (23.80)	31 (73.80)	0.001
Co-morbidities			
Hypertension		36 (85.71)	
IHD		22 (52.38)	
Dyslipidemia		31 (73.80)	
Previous AIS		11 (26.19)	
Duration of T2DM (years)		3.5 ± 1.21	
Diabetes therapy			
Metformin		22 (52.38)	
Non-metformin		20 (47.61)	
Glibenclamide		15 (75.00)	
Pioglitazone		2 (10.00)	
SGT2 inhibitors		3 (15.00)	
Another drug therapy			
Aspirin	8 (38.09)	19 (45.23)	0.59
Clopidogril		11 (26.19)	
Fenofibrate		6 (14.28)	
Statins		9 (21.42)	
β-blockers		31 (73.80)	
CCBs		18 (42.85)	
Antivirals		42 (100)	
Antibiotics		40 (95.23)	
Corticosteroids		20 (47.61)	
Oxygen support		19 (45.23)	
Enoxaparin			
At Covid-19		6 (14.28)	
At AIS		31 (73.80)	

Data presented as n, %, mean ± SD. IHD, ischemic heart disease; AIS, acute ischemic stroke; CCB, calcium channel blocker.

BMI, blood pressure, and the presence of cardiometabolic disturbances according to the American Stroke Association (19).

Statistical Analysis

Data analysis was made by using the Statistical Package for Social Sciences (SPSS) software (IBM Corp, V. 24; Armonk, NY, USA). Results were presented as mean ± standard deviation (SD), absolute values, and percentage. One-way analysis of variance (ANOVA) and unpaired *t* tests were used for the assessment of differences between groups. Pearson correlation was used for calculation of the correlations level. The level of significance was regarded as p value < 0.05.

RESULTS

In the present study, a total of 51 T2DM patients with COVID-19 and AIS were recruited; nine patients were excluded, three due to acute renal failure (ARF), two due to heart failure, and four due to malignancy. Thus, only 42 patients completed the study and were compared to 21 healthy controls. As stated above, patients were divided according to the diabetic pharmacotherapy into metformin-treated ($n = 22$), 52.38%, and non-metformin treated ($n = 20$), 47.61%, groups. Both groups of patients and healthy controls were investigated for the effect of metformin on clinical features and inflammatory and cardiometabolic profiles of T2DM patients with COVID-19 and AIS (**Figure 1**).

TABLE 2 | Clinical presentation of T2DM patients with COVID-19 and acute ischemic stroke.

Clinical findings	Metformin ($n = 22$)	Non-metformin ($n = 20$)	*p* value
Unilateral paralysis	18 (81.81)	20 (100)	0.004
Single limb paralysis	5 (22.72)	3 (15.00)	0.45
Aphasia	9 (40.90)	8 (40.00)	0.94
Dysartharia	11 (50.00)	11 (55.00)	0.7
Dysphagia	4 (18.18)	6 (30.00)	0.31
Delirium	2 (9.09)	2 (10.00)	0.9
Convulsion	3 (13.63)	4 (20.00)	0.53
Visual loss	1 (4.54)	2 (10.00)	0.45
Coma	5 (22.72)	3 (15.00)	0.45
Fever	21 (95.45)	18 (90.00)	0.46
Dyspnea	11 (50.0)	11 (55.00)	0.7
Headache	16 (72.72)	18 (90.00)	0.07
Diarrhea	4 (18.18)	3 (15.00)	0.74
Sweating	19 (86.36)	17 (85.00)	0.88
Anosmia	14 (63.63)	17 (85.00)	0.05

Data presented as n, %.

Regarding patients' demographic features (**Table 1**), there were no differences for both age and gender ($p > 0.05$). The positive family history for T2DM and AIS, as well as smoking status, was higher in AIS patients compared to controls ($p = 0.001$). Concerning the anti-diabetic pharmacotherapy, 52.28% of patients were on metformin therapy and 47.61% were on another diabetic pharmacotherapy, such as glibenclamide, pioglitazone, or sodium-glucose co-transporter (SGT2) inhibitors.

Regarding clinical presentation of T2DM patients with COVID-19, all patients ($n = 42$) presented with AIS, 22 (52.38%) of them were on metformin therapy, and 20 (47.61%) were on another therapeutic regimen. No clinical signs or symptoms significantly differed in T2DM patients with COVID-19 and AIS regarding metformin therapy ($p > 0.05$) with the exception of unilateral paralysis, which was higher in non-metformin (100%) compared to metformin treated patients (100% vs. 81.81%, $p = 0.004$) (**Table 2**).

Concerning the cardiometabolic profile and inflammatory biomarkers in T2DM patients with COVID-19 and AIS (**Table 3**), most parameters were higher in patients compared to controls ($p < 0.001$), although no significant differences in BMI and CRR were stated. In metformin-treated patients, BMI, blood pressure profile, glycemic indices, hemoglobin, and WBC indices did not

TABLE 3 | Cardio-metabolic profile and inflammatory biomarkers in T2DM patients with COVID-19 and AIS regarding metformin therapy compared with the controls.

Variables	Controls ($n = 21$)	Metformin ($n = 22$)	Non-metformin ($n = 20$)	A	B	C	ANOVA
BMI (kg/m^2)	31.68 ± 3.12	31.99 ± 3.71	32.93 ± 3.92	ns	ns	ns	0.51
SBP (mmHg)	123.64 ± 7.36	144.82 ± 9.31	148.89 ± 9.65	0.0001	0.0001	ns	0.0001
DBP (mmHg)	78.56 ± 5.32	87.08 ± 6.47	90.61 ± 7.41	0.0001	0.0001	ns	0.0001
PP (mmHg)	45.08 ± 3.78	57.74 ± 4.92	58.22 ± 5.83	0.0001	0.0001	ns	0.0001
MAP (mmHg)	93.59 ± 7.41	106.33 ± 8.61	110.04 ± 8.46	0.0001	0.0001	ns	0.0001
TC (mg/dL)	147.05 ± 11.68	186.63 ± 9.42	273.52 ± 11.81	0.0001	0.0001	0.0001	0.0001
TG (mg/dL)	144.85 ± 9.51	212.68 ± 13.93	285.42 ± 17.45	0.0001	0.0001	0.0001	0.0001
VLDL (mg/dL)	28.97 ± 3.88	42.53 ± 8.56	57.08 ± 9.47	0.0001	0.0001	0.0001	0.0001
HDL-c (mg/dL)	55.89 ± 4.97	49.22 ± 6.72	40.81 ± 8.22	0.0001	0.0001	0.03	0.0001
LDL-c (mg/dL)	62.20 ± 7.61	94.90 ± 9.52	175.60 ± 11.83	0.0001	0.0001	0.0001	0.0001
CRR	2.63 ± 1.03	3.79 ± 2.61	6.70 ± 4.82	ns	0.004	0.01	0.0004
AI	0.06 ± 0.002	0.27 ± 0.01	0.47 ± 0.02	0.0001	0.0001	0.0001	0.0001
AC	1.63 ± 0.86	2.7 ± 0.96	5.83 ± 1.85	0.02	0.0001	0.0001	0.0001
FBG (mg/dL)	89.63 ± 6.03	144.71 ± 8.91	145.64 ± 8.22	0.0001	0.0001	ns	0.003
HbA1c (%)	5.3 ± 0.53	6.7 ± 2.41	7.4 ± 2.82	0.04	0.01	ns	0.007
Serum insulin (mIU/L)	8.13 ± 2.11	17.52 ± 3.81	18.56 ± 3.25	0.01	0.01	ns	0.003
HOMA-IR	1.63 ± 0.86	6.92 ± 2.61	7.04 ± 3.61	0.003	0.003	ns	0.002
S. Ferritin (ng/mL)	90.51 ± 10.87	365.93 ± 17.41	475.92 ± 22.78	0.0001	0.0001	0.0001	0.0001
CRP (mg/L)	3.16 ± 1.08	43.85 ± 6.04	48.54 ± 6.13	0.0001	0.0001	0.04	0.0001
LDH (U/L)	133.71 ± 9.53	287.92 ± 13.84	299.63 ± 14.75	0.0001	0.0001	0.0001	0.0001
D-dimer (ng/mL)	21.92 ± 3.17	285.53 ± 14.94	307.84 ± 15.82	0.0001	0.0001	0.0001	0.0001
Oxygen saturation (%)	98.99 ± 1.11	94.86 ± 3.61	94.62 ± 3.79	0.0001	0.0001	ns	0.0001
WBC ($10^3/\mu L$)	8.30 ± 2.16	16.63 ± 3.85	17.92 ± 3.44	0.0001	0.0001	ns	0.0001
Neutrophil %	66.53 ± 4.81	77.42 ± 8.21	78.66 ± 8.11	0.0001	0.0001	ns	0.0001
Lymphocyte %	34.86 ± 6.92	16.58 ± 5.49	14.87 ± 4.31	0.0001	0.0001	ns	0.0001
Hb (mg/dL)	13.59 ± 1.43	12.53 ± 1.22	12.74 ± 1.64	0.04	ns	ns	0.04
Platelets ($10^3/\mu L$)	342.69 ± 12.94	262.43 ± 12.49	209.45 ± 11.82	0.0001	0.0001	0.0001	0.0001

Data presented as mean ± SD. ns, not significant; A, metformin vs. control; B, non-metformin vs. control; C, metformin vs. non-metformin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, Pulse pressure; MAP, mean arterial pressure; TG, total triglyceride; TC, total cholesterol (TC); HDL, high density lipoprotein; LDL, low density lipoprotein; AI, atherogenic index; VLDL, very low density lipoprotein; AI, atherogenic coefficient; CRR, cardiac risk ratio; FBG, Fasting blood glucose; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic model for assessment of insulin resistance; LDH, lactate dehydrogenase.

differ from that stated in non-metformin treated patients ($p > 0.05$). On the other hand, lipid profile, atherogenic indices, and inflammatory cytokines were lower in metformin-treated patients as compared to non-metformin treated patients ($p < 0.05$). Ferritin serum levels were lower in metformin-treated when compared to non-metformin treated patients (365.93 ± 17.41 ng/mL vs. 475.92 ± 22.78 ng/mL, $p = 0.0001$). In addition, the serum CRP levels were lower in metformin-treated when compared to non-metformin treated patients ($p = 0.04$). Both LDH and D-dimer serum levels were lowered in metformin-treated patients compared to non-metformin treated patients ($p = 0.0001$). Platelet count was reduced in non-metformin treated patients compared to metformin treated patients ($p = 0.0001$).

In addition, lung CT scan score percentage of COVID-19 patients was 30.62 ± 10.64 for metformin-treated patients and 36.31 ± 5.03 for non-metformin treated patients (**Figure 2**). Moreover, the neutrophil: lymphocyte ratio (NLR) was higher in T2DM patients with COVID-19 and AIS compared to controls ($p = 0.0001$); however, NLR did not significantly differ between non-metformin treated and metformin-treated patients (5.2 ± 1.2 vs. 4.67 ± 1.7, $p = 0.06$) (**Figure 3**).

Also, the SRR in T2DM patients with COVID-19 and AIS was higher in comparison to controls ($p = 0.0001$), although was lower in metformin-treated patients (3.42 ± 1.05) when compared to non-metformin treated patients (7.96 ± 1.96) ($p = 0.01$) (**Figure 4**). Also, SRR was correlated with the inflammatory biomarkers in T2DM patients with COVID-19 and AIS in both metformin-treated and non-metformin treated patients ($P < 0.001$) (**Table 4**).

DISCUSSION

In this study, it was illustrated that SARS-CoV-2 infection in T2DM patients augments the risk of AIS through the provocation of underlying cardio-metabolic disturbances, as reported by Zaki et al. (20). Most recruited patients had a positive family history of stroke, smoking, hypertension, T2DM, and dyslipidemia that

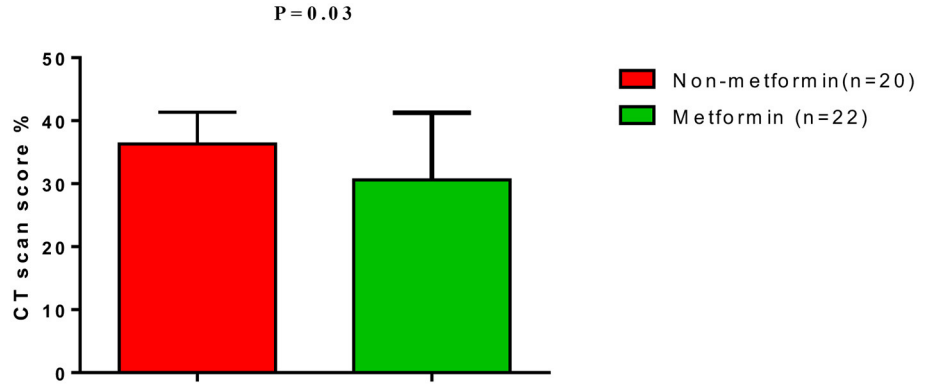

FIGURE 2 | Lung CT scan score in T2DM patients with COVID-19 on metformin therapy.

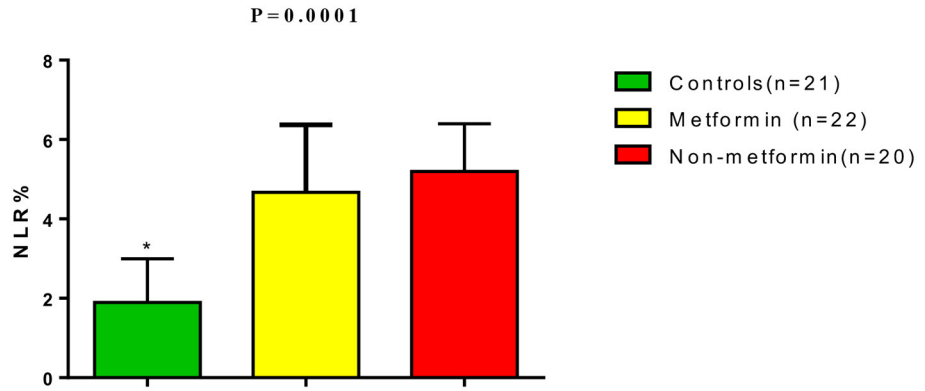

FIGURE 3 | Neutrophil: lymphocyte ratio (NLR) in T2DM patients with COVID-19 and AIS compared with the controls. *$P = 0.0001$ compared to metformin and non-metformin treated patients.

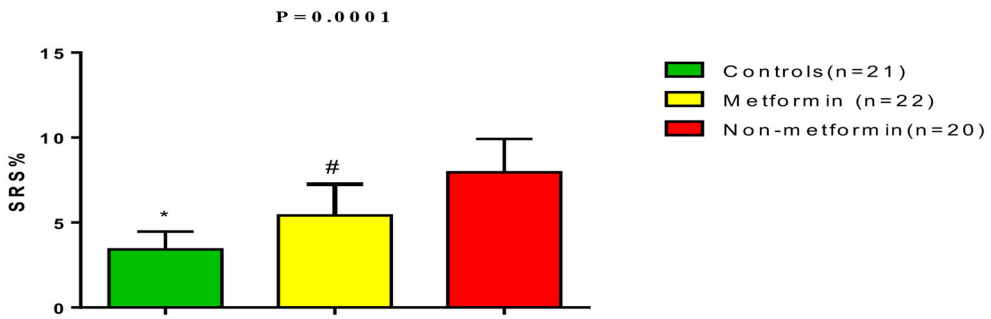

FIGURE 4 | Stroke risk ratio (SRR) in T2DM patients with COVID-19 and acute ischemic stroke compared with the controls. *$P = 0.0001$ compared to metformin and non-metformin treated patients. #$P = 0.01$ compared non-metformin treated patients.

increased the risk of AIS during the development of COVID-19, as confirmed by Saban-Ruiz et al. (21). Correspondingly, it has been shown that the risk of AIS during COVID-19 is approximately 5%, with 65.6% of AIS in COVDI-19 patients being labeled as cryptogenic stroke. A delay in the diagnostic investigation of COVID-19 patients may also contribute to the high rate of cryptogenic stroke (22).

In our retrospective cohort study, the clinical presentation of the disease started within 1 week after SARS-CoV-2 infection, as reported by Yaghi et al. (23) who stated in their retrospective cohort study a median of 10 days for AIS onset following COVID-19. COVID-19-induced-AIS may be due to a combination of cardio-metabolic disturbances and COVID-19-related mechanisms, such as hyper-coagulation,

TABLE 4 | Correlation of inflammatory biomarkers in T2DM patients with COVID-19 and acute ischemic stroke.

Biomarkers	Metformin ($n = 22$)		Non-metformin ($n = 20$)	
	r	p	r	p
S. Ferritin (ng/mL)	0.56	0.006	0.77	0.0001
CRP (mg/L)	0.61	0.002	0.84	0.0001
LDH (U/L)	0.58	0.004	0.75	0.0001
D-dimer (ng/mL)	0.91	0.0001	0.96	0.0001
NLR%	0.55	0.005	0.56	0.006

NLR, neutrophil: lymphocyte ratio; LDH, lactate dehydrogenase.

cytokine storm, vasculitis, endothelial dysfunction, and cardiomyopathy-induced cardiac arrhythmias (24). Notably, binding of SARS-CoV-2 to the ACE2 receptors, which are also abundant in the vascular endothelium, may trigger an inflammatory reaction and lymphocytic endothelitis with subsequent development of endothelial dysfunction and overall impairment of microcirculatory function (25). In addition, the endothelial dysfunction induced by SARS-CoV-2, along with the underlying T2DM-induced endothelial dysfunction, leads to cerebral microcirculatory failure with the development of brain ischemia and AIS (26). Thereby, the development of AIS in the T2DM patients of the present study might be due to a the pre-existence of cardio-metabolic disorders during the development of COVID-19 pneumonia.

On the other hand, data obtained in this study also underline the association between COVID-19 and elevation of inflammatory (CRP and ferritin), coagulation (D-dimer), and tissue injury biomarkers (LDH), consistent with recent published studies (27). Indeed, hyperinflammation and cytokine storm in COVID-19 increase the risk of multi-focal AIS due to impairment of vasodilator endothelial heparin sulfate, complement activation, and micro-vascular thrombosis (27). For example, a recent study comparing AIS alone with COVID-19-associated AIS showed no differences in cardio-metabolic risk factors, suggesting a specific mechanism of SARS-CoV-2 action in the pathogenesis of AIS (28). However, in the present study most recruited patients had a poor cardio-metabolic profile that predisposed them for the development of AIS. So, the pure mechanism of SARS-CoV-2-induced-AIS was not confirmed in our study due to limitations in the detection of SARS-CoV-2 at the infarction sites.

Indeed, SARS-CoV-2 binding to the ACE2 receptor in cardiomyocyte leads to myocardial injury with the induction of cardiac arrhythmia and increasing risk of thrombo-embolism-induced AIS (29). Respiratory insufficiency-induced hypoxemia and cytokine storm in COVID-19 also cause indirect cardiomyocyte injury (30). However, the echocardiographic profile and troponin serum levels were not addressed in this study.

Furthermore, in this study, 14.28 and 73.80% of recruited patients received enoxaparin at the time of COVID-19 and AIS diagnosis, respectively. Indeed, a delay in the initiation and the use of unstandardized enoxaparin therapy may increase both COVID-19 severity and complications, since enoxaparin therapy reduces the risk of thrombo-embolism and development of AIS (31). Also, enoxaparin improves the clinical outcomes of COVID-19 patients, as it exerts a remarkable anti-inflammatory and antiviral effect that attenuates COVID-19 coagulation disorders, which are found in 22–55% of hospitalized patients (32). In the present study, the high D-dimer serum level in the recruited patients reflects the underlying COVID-19 induced-coagulation disorders.

Specifically looking at the core of the present context, which was to address whether metformin improved clinical and laboratory findings in COVID-19 patients when compared with non-metformin treated COVID-19 patients, a lower percentage of unilateral paralysis in metformin-treated patients when compared to non-metformin treated patients was shown. Similar findings were also disclosed by Mima et al. (33), who showed that metformin therapy in T2DM patients with AIS reduces both acute neurological deficit and severity. Further, the findings obtained here illustrate that metformin therapy in T2DM patients with COVID-19 and AIS was associated with a better cardiometabolic profile and inflammatory cytokines levels compared with non-metformin-treated patients, mostly attributed to their anti-inflammatory effects and ability to improve the IR. Similarly, Kow and his colleague (34) found that metformin therapy is associated with a reduction in the mortality rate in hospitalized COVID-19 patients with T2DM due to its anti-inflammatory and antiviral effects. Indeed, it has been stated that the anti-inflammatory effect of metformin is through the activation of the AMPK pathway that inhibits the mTOR signaling and NF-kB pathway with subsequent suppression of IL-6 and TNF-α with the activation of anti-inflammatory IL-10. These changes triggered by metformin are also able to attenuate the cytokine production from activated macrophage and glial cells during severe COVID-19 and AIS-induced neuroinflammation (35). Zeng et al. (36) confirmed that metformin has a neuroprotective effect via the inhibition of apoptosis and oxidative stress in AIS and thus can be viewed as a promising preventive agent against ischemic-reperfusion in AIS. However, biomarkers of oxidative stress and cellular activity of NF-kB and/or AMPK pathways were not evaluated in the present study in relation to metformin therapy (37, 38).

Furthermore, the NLR was higher in T2DM patients with AIS and COVID-19 compared to the healthy controls due to neutrophil activation and lymphopenia. In fact, both AIS and COVID-19 are associated with high NLR, with a high NLR in AIS being linked to poor neurological outcomes and risk of intra-cerebral hemorrhage (39), while high NLR in COVID-19 patients is regarded as an independent risk factor for COVID-19 hospitilization and associated with a high mortality rate (40). Dermirdal et al. (41) also found that metformin reduces NLR in patients with T2DM. From this, it can be seen that neutrophils play a fundamental role in the inflammatory responses to ischemia/reperfusion injury by releasing oxidants, proteases, toll like receptors (TLRs) activation, and releasing inflammatory products (42). Despite these findings, metformin therapy in

the present study was not associated with a reduction of NLR, probably due to the small sample size or the dosage amount of metformin not being sufficient to overcome neutrophil recruitment. Soraya et al. (43) confirmed the dose-dependent effect of metformin in the reduction of neutrophil activation and recruitment.

Of note, metformin therapy has been linked to a lower SRS compared to non-metformin treated patients due to its neuroprotective effects (39). Metformin pre-treatment with metformin in T2DM patients has also been associated with reduced neurological severity during AIS development (44). The potential neuroprotective effect of metformin in AIS has also been related to an AMPK dependent-inhibition of the NFκB pathway, cytokine activation, and associated blood brain barrier disruption with significant amelioration of neuronal glucose-oxygen consumption (33). Venna et al. (45) confirmed that metformin therapy improves post-stroke recovery through the modulation of AMPK signaling. Nevertheless, post-stroke outcomes were not addressed in the present study.

Undeniably, the present study illustrated that metformin therapy was linked to a lower ALI in COVID-19 patients, as revealed by a recent report (45). However, although Do et al. (46) revealed an insignificant effect of metformin on the amelioration of ALI in T2DM patients with COVID-19, Wu et al. (47) confirmed that metformin therapy may alleviate the endotoxemia-induced ALI through restoration of lung AMPK dependent inhibition of mTOR signaling. Thus, metformin therapy in T2DM patients with COVID-19 and AIS leads to dual protective effects on both the lung and brain.

On the other hand, metformin has potential antiviral effects against different viruses through the activation of the AMPK pathway (47). Recently, different studies have confirmed the antiviral effect of metformin against SARS-CoV-2 replication (48). The anti-SARS-CoV-2 activity of metformin is related to different mechanisms, namely the AMPK activation by metformin that leads to phosphorylation of the ACE2 receptor at Ser-680, where the interaction, stabilization, and conformational changes of ACE2 occur. These changes prolong the half-life of bound and soluble ACE2 and become less sensitive for SARS-CoV-2 binding (35). Also, the protective role of metformin against ALI in COVID-19 patients seems to be related to lung ACE2 up-regulation, exerting both anti-inflammatory and anti-apoptotic effects. Additionally, up-regulated ACE2 prevents the deleterious effect of high Ang II level in COVID-19-induced pneumonia (49). Dalan (50) found that metformin mitigates ALI in COVID-19-induced pneumonia through the inhibition of neutrophil migration and chemotaxis with a mast cell stabilizing effect.

Therefore, despite the small sample size, this study discloses for the first time the dual protective effect of metformin against COVID-19-induced pneumonia, ALI, and AIS through amelioration of hyper-inflammation and underlying cardiometabolic disturbances.

This study also has several limitations, the first one related to the small sample size and the second one related to the retrospective nature, so that long-term outcomes were not determined. In addition, gender differences were not evaluated since most recruited patients were males. The level of soluble ACE2 serum level was also not determined. Nevertheless, due to its strengths, this should be considered a preliminary study to trigger future large-scale prospective studies to confirm the link between ALI and AIS in DM patients with COVID-19.

CONCLUSION

Metformin therapy in T2DM patients was associated with a lower risk of AIS during COVID-19. Further studies are needed to observe the link between AIS in COVID-19 diabetic patients under metformin therapy. However, we cannot draw any ultimate conclusions from our observation due to the small sample size. Therefore, we hypothesized that metformin therapy may attenuate and treat Covid-19 and associated AIS and ALI, prospective, randomized controlled studies are recommended in this regard.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research and Ethical Committee Board, College of Medicine, Al-Mustansiryia University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SYSTEMATIC REVIEW
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Risk of Clinically Relevant Venous Thromboembolism in Critically Ill Patients With COVID-19: A Systematic Review and Meta-Analysis

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Background: Early during the course of the ongoing COVID-19 pandemic, reports suggested alarmingly high incidences for thromboembolic events in critically ill patients with COVID-19. However, the clinical relevance of these events was not reported in several studies. Additionally, more recent research showed contradictory results and suggested substantially lower rates of venous thromboembolism. Thus, the aim of the present study was to summarize evidence on the incidence of clinically relevant venous thromboembolism (VTE)—defined as VTE excluding isolated subsegmental pulmonary embolism (PE) and distal deep vein thrombosis (DVT)—in adult critically ill patients with COVID-19.

Methods: We performed a systematic review of studies reporting the incidence of clinically relevant PE and/or DVT in critically ill patients with COVID-19. Scientific reports published in the English language between January and October 2020 were included. We conducted a random-effects model meta-analysis to calculate incidence estimates of clinically relevant VTE and bleeding events. We also performed exploratory meta-regression and subgroup analyses of different diagnostic approaches and additional factors that possibly influenced the incidence of these outcomes.

Results: Fifty-four articles (5,400 patients) fulfilled the predefined inclusion criteria, of which 41 had a high risk of bias. The majority of included patients were male, > 60 years, and overweight. Twenty-one studies reported the use of prophylactic doses of heparin. Pooled incidences for clinically relevant PE were estimated at 8% (95% CI, 4–11%), for proximal DVT at 14% (95% CI, 9–20%), and—after exclusion of studies with a high risk of bias—for the composite outcome of VTE at 18% (95% CI, 13–24%). Clinically relevant bleeding occurred at a rate of 6% (95% CI, 2–9%).

Conclusions: We summarized currently available data on the rate of clinically relevant VTE in critically ill patients with COVID-19. Pooled incidence estimates were lower than those reported by previous review articles. In the absence of evidence-based

anticoagulation guidelines for critically ill patients with COVID-19, the results of our study provide clinically important information for an individual risk-benefit assessment in this context.

Registration: The study protocol was prospectively registered in PROSPERO on June 22, 2020 (CRD42020193353; https://www.crd.york.ac.uk/prospero).

Keywords: venous thromboembolism, COVID-19, incidence, pulmonary embolism, deep vein thrombosis, critically ill patients

INTRODUCTION

The COVID-19 pandemic has spread globally since the beginning of 2020, with ∼74.5 million confirmed cases and >1.6 million deaths worldwide as of December 21, 2020 (1). SARS-CoV-2 infection has been linked to a wide spectrum of clinical presentations, ranging from mild courses to critical illness (2). A number of publications have indicated that in a subset of patients with COVID-19, coagulopathy could complicate the course of disease and might have an impact on mortality (3–5). For critically ill patients with COVID-19 in particular, early reports suggested an alarmingly high incidence of thromboembolic events of up to 69% (6). However, these numbers have been contradicted by more recent publications, reporting radiographically confirmed venous thromboembolism (VTE) in 8% of critically ill patients with COVID-19 (7).

It has been decades since VTE—defined as the occurrence of deep vein thrombosis (DVT) or pulmonary embolism (PE)—was recognized as a common and potentially fatal complication in critically ill patients (8). Accordingly, current guidelines strongly recommend the use of pharmacological thromboprophylaxis for all critically ill patients without contraindications (9–11).

Hence, it was not surprising when, early in the course of the pandemic, Tang et al. reported a decrease in mortality in patients with COVID-19 with the use of anticoagulant treatment (12). Meanwhile, a number of interim guidance documents on the coagulation management of hospitalized patients with COVID-19 have emerged. Some authors recommend the use of high-prophylactic doses of heparin (13), whereas others suggest that higher doses should be considered in critically ill patients (14), although the results of large-scale clinical trials comparing the use of different anticoagulant regimens in critically ill patients with COVID-19 are still pending (15). However, bleeding has been identified as a relevant risk in critically ill patients (16), and the use of higher doses of antithrombotic agents might further aggravate this risk. Recent publications highlight that the ideal dose of anticoagulants still remains unclear (17).

To better understand these conflicting data and to inform evidence-based guidelines for clinicians, it is important to assess reliable data on the incidence of *clinically relevant* VTE and of bleeding episodes in patients with COVID-19. Thus, the aim of this systematic review was to provide robust estimates of clinically relevant VTE incidence rates in adult critically ill patients with COVID-19 together with estimates of bleeding rates.

METHODS

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (18). The study protocol was prospectively registered in PROSPERO (CRD42020193353).

Literature Search and Study Selection

MEDLINE (via OVID), Embase, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science were searched by a dedicated librarian (EC) to identify studies published between January 1, 2020, and October 7, 2020. The detailed search strategy is provided in **Additional File 1**. In addition, the bibliographies of the included articles were searched by hand.

After deduplication of the search results, titles and abstracts were screened in duplicate for potential relevance by two independent investigators (JG, MW). Interventional and retrospective or prospective observational studies reporting the incidence of radiographically confirmed VTE (i.e., DVT and/or PE) in adult critically ill patients with COVID-19 were included. Studies reporting VTE rates in preselected patient cohorts undergoing specific diagnostic procedures rather than a collective of critically ill patients were excluded. Similarly, postmortem studies were excluded. Furthermore, reports in any language other than English were excluded. Publications judged to be potentially relevant underwent a full-text assessment to determine inclusion by two independent investigators (JG, MW). Disagreements on study eligibility were resolved by consensus or adjudication by a third investigator (ES).

Data Extraction and Outcomes

Data were extracted into a predefined form in duplicate by two independent investigators (JG, MW). Disagreements were resolved by consensus or adjudication by a third investigator (ES). Extracted data included (i) study details (e.g., study design, publication date, institutional review board (IRB) approval), (ii) patient characteristics (e.g., number of included patients, age, body mass index), (iii) predefined outcomes (e.g., DVT, PE, overall VTE rate), and (iv) potential confounders (e.g., active cancer, duration of disease, type of anticoagulation).

Primary outcomes of interest were the incidence of (i) clinically relevant and radiographically confirmed PE and (ii) clinically relevant and radiographically confirmed DVT. We judged PE to be clinically relevant when the deterioration of patients' conditions close to the time of diagnosis was reported

(e.g., abrupt hemodynamic and/or respiratory deterioration led to a radiographic examination confirming the diagnosis of PE). Additionally—and specifically if details of the patients' conditions were lacking—we subtracted the number of reported cases of isolated subsegmental PE from the overall number of reported cases of PE. With regard to DVT, as an approximation of clinical relevance, we subtracted the number of reported cases of isolated distal DVT from the overall number of reported cases of DVT. With the same intent, we performed a subgroup analysis according to whether routine ultrasound screening was performed to detect DVT. We did not include catheter-related thrombosis in the definition of DVT.

Secondary outcomes included the overall number of any form of PE, the overall number of any form of DVT, and the composite outcome of any form of VTE. Furthermore, the rate of clinically relevant bleeding events (including intracranial bleeding as a subcategory) was determined.

Additionally, we extracted the number of computed tomography (CT) scans performed. Type of anticoagulation was categorized as none, standard (= high-risk prophylaxis) dose heparin, high-dose heparin, any dose heparin, or other forms of anticoagulation. We did not differentiate between the use of low-molecular-weight heparin or unfractioned heparin because the majority of studies did not provide this information.

Quality Assessment

Currently, there is no available standardized risk of bias assessment tool for incidence or prevalence studies (19). Therefore, we evaluated three different tools in a pilot examination of five studies performed by two independent investigators (JG, MW): the tool developed by Hoy et al. the Joann Briggs Institute Critical Appraisal Checklist for Prevalence Studies, and ROBINS-i (19–21). The tool developed by Hoy et al. was found to have the highest interrater reliability and was thus subsequently used for the quality assessment of the included studies. Briefly, it focuses on five factors determining external validity and five factors determining internal validity using 10 questions. When applicable, questions covering internal validity were answered separately for the outcomes of PE and DVT. When the lack of details provided in a study prevented the answering of a question, the respective item was determined to have a high risk of bias. A final summary item for the overall risk of bias identified studies as having a low, moderate, or high risk of bias. Quality assessment was performed in duplicate by two independent investigators (JG, MW), and disagreements were resolved by consensus or adjudication by a third investigator (HH). The risk of bias assessment was performed only with regard to the relevant outcomes for the present meta-analysis and did not judge the overall quality of included studies.

Statistical Analysis

We conducted a random-effects model meta-analysis to calculate pooled estimate incidence rates and 95% confidence intervals (CI 95%) for the following four predefined outcomes: (i) PE, (ii) DVT, (iii) VTE, and (iv) bleeding episodes. We corrected for clinically relevant types of PE by calculating the pooled incidence rates of non-subsegmental PE. Similarly, we corrected for clinically relevant types of DVT by calculating the pooled incidence rates of proximal DVT. Additionally, we performed exploratory random-effects meta-regression and subgroup analyses for a number of factors that possibly influenced the estimated incidence rates, including (i) different diagnostic approaches (ultrasound screening for DVT, proportion of patients undergoing CT scans), (ii) quality of the included studies, (iii) date of publication, (iv) sample size of included studies, and (v) different anticoagulation regimens. The heterogeneity of included trials is reported using I^2. To account for small-study effects, zero-event studies were not included in the main analysis. However, a sensitivity analysis was carried out that included zero-event studies using a mixed-effects model to calculate pooled estimate incidences and 95% CI. For each investigated outcome, forest plots were produced. Each meta-regression was visualized using bubble plots. Microsoft Excel (Microsoft, Redmond, WA, USA), Python (Version 3), and Stata (Version 16, College Station, TX, USA) were used for data management, statistical analyses, and graph production.

RESULTS

Study and Patient Characteristics

The literature search yielded 5,215 results, of which 54 were deemed eligible for inclusion (6, 7, 22–73). **Figure 1** presents the process used for the identification, screening, and inclusion of articles.

The included studies reported on a total of 5,400 critically ill patients with COVID-19 from four different continents (Asia, Europe, North America, South America). Detailed characteristics of the studies are shown in **Supplementary Table 1**. The majority of the included studies reported retrospectively collected data, whereas two studies were prospective, interventional trials. Of note, four studies did not report having obtained IRB approval, and two studies explicitly stated not having sought IRB approval. The number of included patients per study ranged from 16 to 829 patients. Sample sizes for the extracted outcomes—the denominators—ranged from 1,074 patients (secondary outcome of intracranial bleeding) to 5,400 patients (composite outcome of VTE). Regarding the quality assessment, 41 of the studies were found to have a high risk of bias, whereas 13 studies were deemed to carry a moderate risk of bias. None of the included studies were judged to have a low risk of bias with regard to reporting the relevant outcomes. **Supplementary Table 2** shows the detailed results of the quality assessment of the included studies.

Supplementary Table 3 shows the relevant patient characteristics, including possible confounders regarding VTE incidence, as well as the thromboprophylactic or anticoagulant regimen for each included study. A substantial number of studies did not report relevant confounding parameters, such as age, body mass index (BMI), length of stay in the intensive care unit (ICU LOS), or disease duration. Studies that reported detailed patient characteristics included a largely comparable patient collective. The overall trend in these studies was that the majority of patients were male, with an advanced age $>$ 60 years, and overweight. Thirteen studies reported an average BMI $\geq$ 30 kg/m^2. With regard to thromboprophylaxis and anticoagulation, 21 studies reported using a prophylactic standard heparin dose,

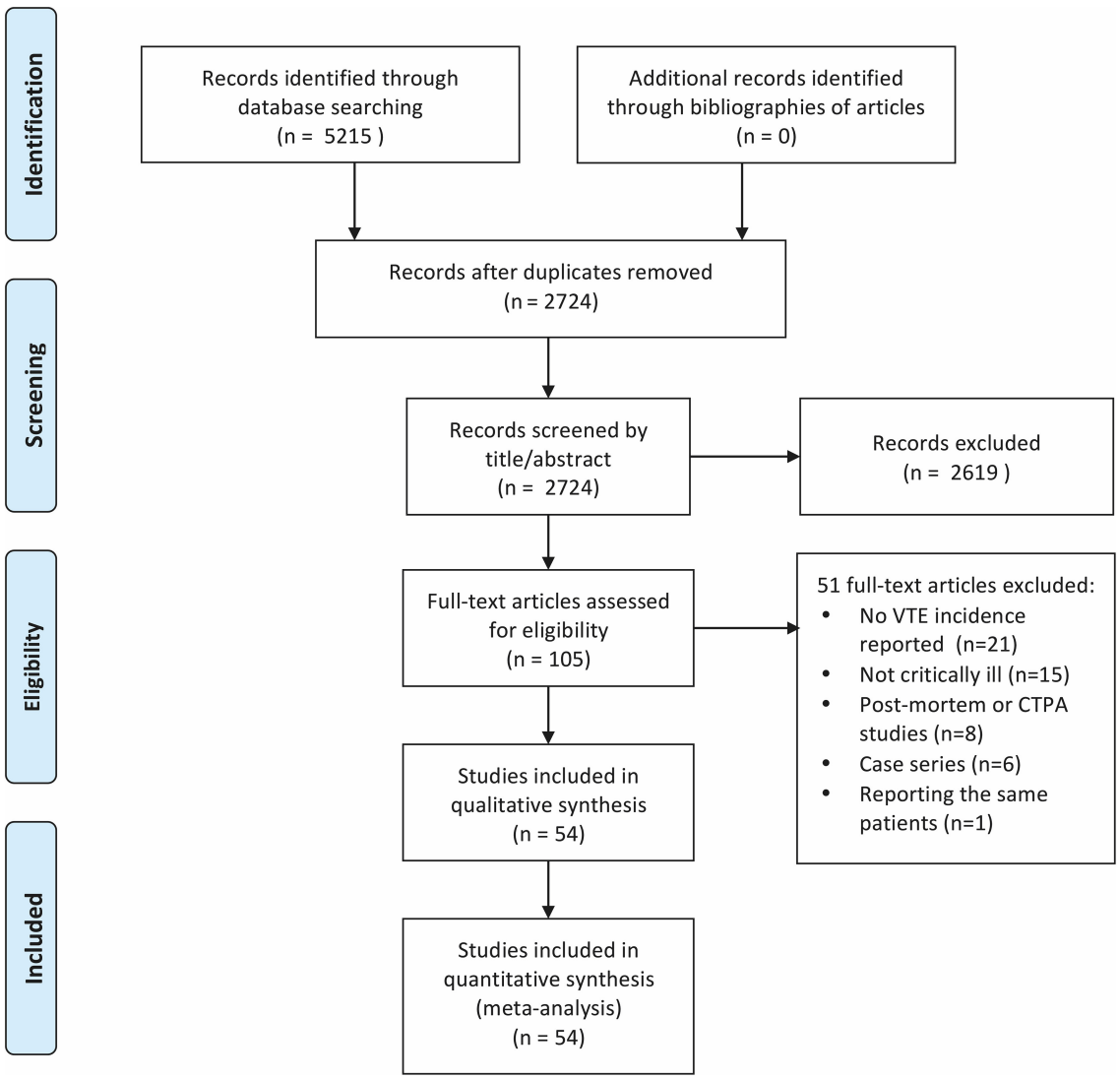

FIGURE 1 | PRISMA flowchart showing the process from identification to inclusion of articles.

whereas 24 studies reported the use of mixed or higher doses of heparins. Five studies reported using other anticoagulant substances, one study explicitly reported not having used thromboprophylaxis at all, and three studies did not provide details about the form of anticoagulation.

Primary Outcomes: Clinically Relevant PE and DVT

Ten studies provided enough information to extract data on the occurrence of ***clinically relevant PE***. The pooled incidence of clinically relevant PE was 8% (95% CI, 4–11%), with a substantial heterogeneity among studies ($I^2 = 68\%$, **Figure 2A**). Exclusion of subsegmental forms of PE was possible in ten studies, resulting in a pooled incidence of 12% (95% CI, 7–16%) for non-subsegmental PE and a considerable heterogeneity among studies ($I^2 = 87\%$, **Figure 2B**).

Fourteen studies provided enough information to subtract cases of isolated distal DVT from the total number of reported cases of DVT. The pooled incidence of ***proximal DVT*** was 14% (95% CI, 9–20%, **Figure 3**). Heterogeneity among studies was considerably high ($I^2 = 91\%$).

Thirty-three studies provided information on whether routine ultrasound screening for DVT was performed. Subgroup analysis resulted in a pooled incidence of 10% (95% CI, 6–14%, **Figure 4**) for 15 studies that did not perform screening. In contrast, studies that included ultrasound screening results were determined to have a pooled incidence of 38% (95% CI, 28–48%, **Figure 4**).

Secondary Outcomes and Additional Subgroup Analyses

In total, 39 studies reported on the occurrence of ***any form of PE***, resulting in a pooled incidence of 12% (95% CI, 6–17%) for studies judged to have a moderate risk of bias, whereas the pooled incidence for studies with a high risk of bias was 13% (95%

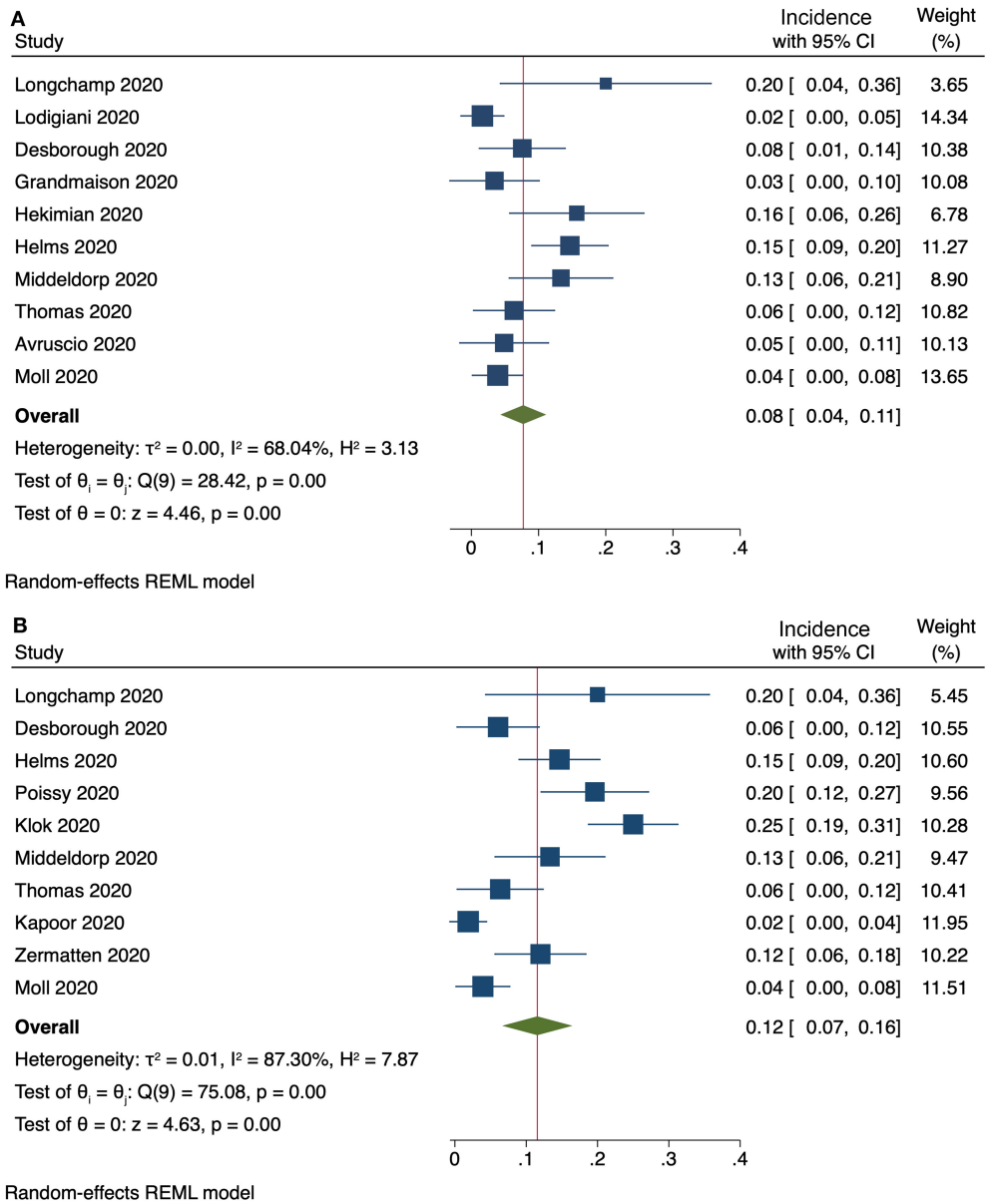

FIGURE 2 | (A) Incidence of clinically relevant PE (10 studies) and **(B)** incidence of non-subsegmental PE (10 studies).

CI, 10% to 13%; **Supplementary Figure 1**). Heterogeneity was considerably higher ($I^2 = 90\%$) than for the outcome parameter of *clinically relevant PE*.

Sixteen studies reported the number of computer tomography (CT) scans performed to detect PE. In total, 514 CT scans were obtained for 1,433 patients. There was a strong positive correlation between the proportion of patients who underwent CT scans and the incidence of any form of PE ($R^2 = 69\%$, $p < 0.001$, **Supplementary Figure 2A**). In contrast, we did not find a correlation between the proportion of patients who underwent CT scans and the rate of non-subsegmental PE ($R^2 = 14\%$, $p = 0.23$, **Supplementary Figure 2B**).

Overall, the occurrence of ***any form of DVT*** was reported in 40 studies. Studies judged to have a moderate risk of bias were determined to have a pooled incidence of 11% (95% CI, 6–16%), whereas studies with a high risk of bias were determined to have an incidence of 26% (95% CI, 18–34%, **Supplementary Figure 3**).

The pooled incidence of ***any form of VTE***, a composite outcome of any form of PE and DVT, was 18% (95% CI, 13–24%) in studies with a moderate risk of bias (**Figure 5**). In contrast, studies judged to have a high risk of bias were found to have a pooled VTE incidence of 31% (95% CI, 24–37%). One additional study explicitly reported not having observed VTE in the included patient cohort. Including this study in a

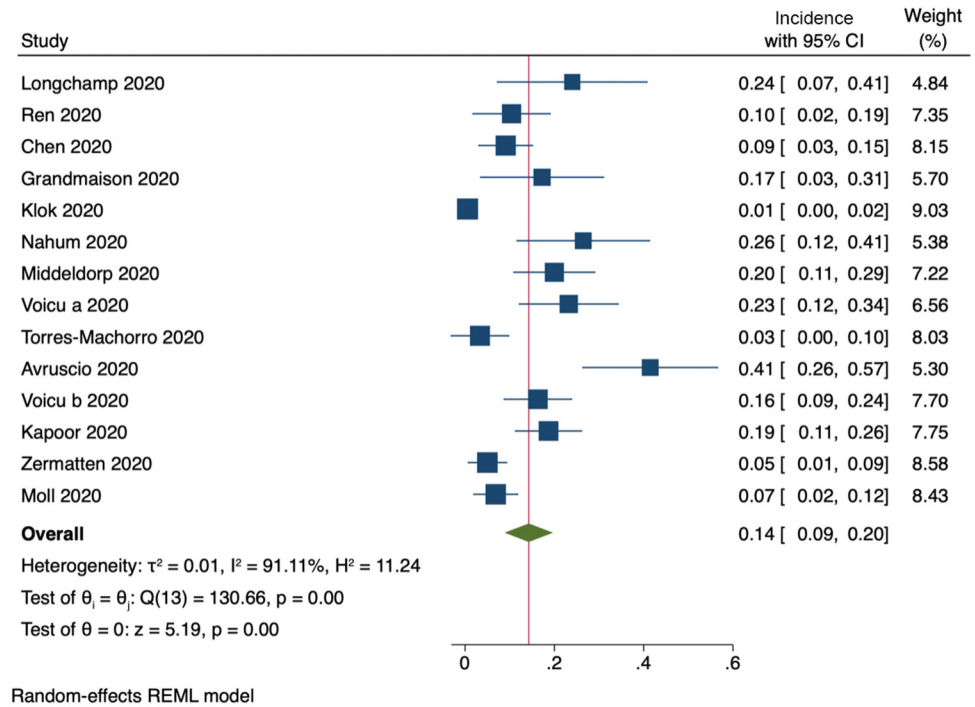

FIGURE 3 | Incidence of proximal DVT (14 studies).

sensitivity analysis using a mixed-effects model resulted in an overall incidence rate of 22% (95% CI, 16–28%).

Routine ultrasound screening was associated with an increase in the reported VTE incidence (**Supplementary Figure 4**), whereas larger study sample sizes showed a trend toward lower VTE incidence rates (**Supplementary Figure 5**). We did not observe differences in VTE incidence rates associated with the date of publication (**Supplementary Figure 6**). **Supplementary Figure 7** shows no difference in pooled incidences of VTE for different subgroups according to anticoagulant regimen.

Eleven studies included information on the incidence of ***clinically relevant bleeding*** events (**Figure 6**). The pooled incidence was 6% (95% CI, 2–9%). Furthermore, six studies reported on the incidence of intracranial bleeding, with a pooled incidence of 2% (95% CI, 0.6–2.4%).

DISCUSSION

To the best of our knowledge, the current study is the first systematic review and meta-analysis to focus not only on the crude incidence but on the *clinical relevance* of VTE in critically ill patients with COVID-19. Current guidelines recognize risks associated with a potential overdiagnosis of incidental subsegmental PE and distal DVT that might be of questionable clinical relevance (74, 75). Therefore, we extracted data on the rates of clinically relevant PE and proximal DVT and examined the possible influence of different diagnostic approaches on the reported incidences. We found incidence estimates of 8, 14, and 18% for PE, DVT, and VTE, respectively. We could demonstrate the substantial influence of a high rate of CT scans and routine ultrasound screening on reporting higher incidences of isolated subsegmental PE and isolated distal DVT, respectively. Furthermore, we are the first to report a pooled incidence rate of 6% for clinically relevant bleeding and of 2% for intracranial bleeding in this specific patient cohort.

Overall, the included studies reported on a patient group with a collective high baseline risk of VTE. Patients were critically ill and thus bedridden, with reported mean ICU LOS considerably longer than 7 days. In studies that provided detailed patient characteristics, the majority of patients were at an advanced age and overweight. In addition to critical illness, immobility, advanced age and obesity have all previously been associated with increased VTE risk (76). VTE has thus long been recognized as a serious problem in critically ill patients (77). Hence, the VTE incidence reported in the current study needs to be viewed primarily in light of these relevant background factors and not only the COVID-19 disease. Additionally, infection *per se* is another risk factor for the occurrence of VTE. Severe COVID-19 disease is accompanied by excessive cytokine release, which in turn activates the coagulation cascade, resulting in typical laboratory alterations such as elevated fibrinogen and D-dimer levels (78). The close connection between inflammation and coagulation—immunothrombosis—has been known for more than a century (79). It thus seems reasonable to compare our findings with VTE rates in critically ill patients with

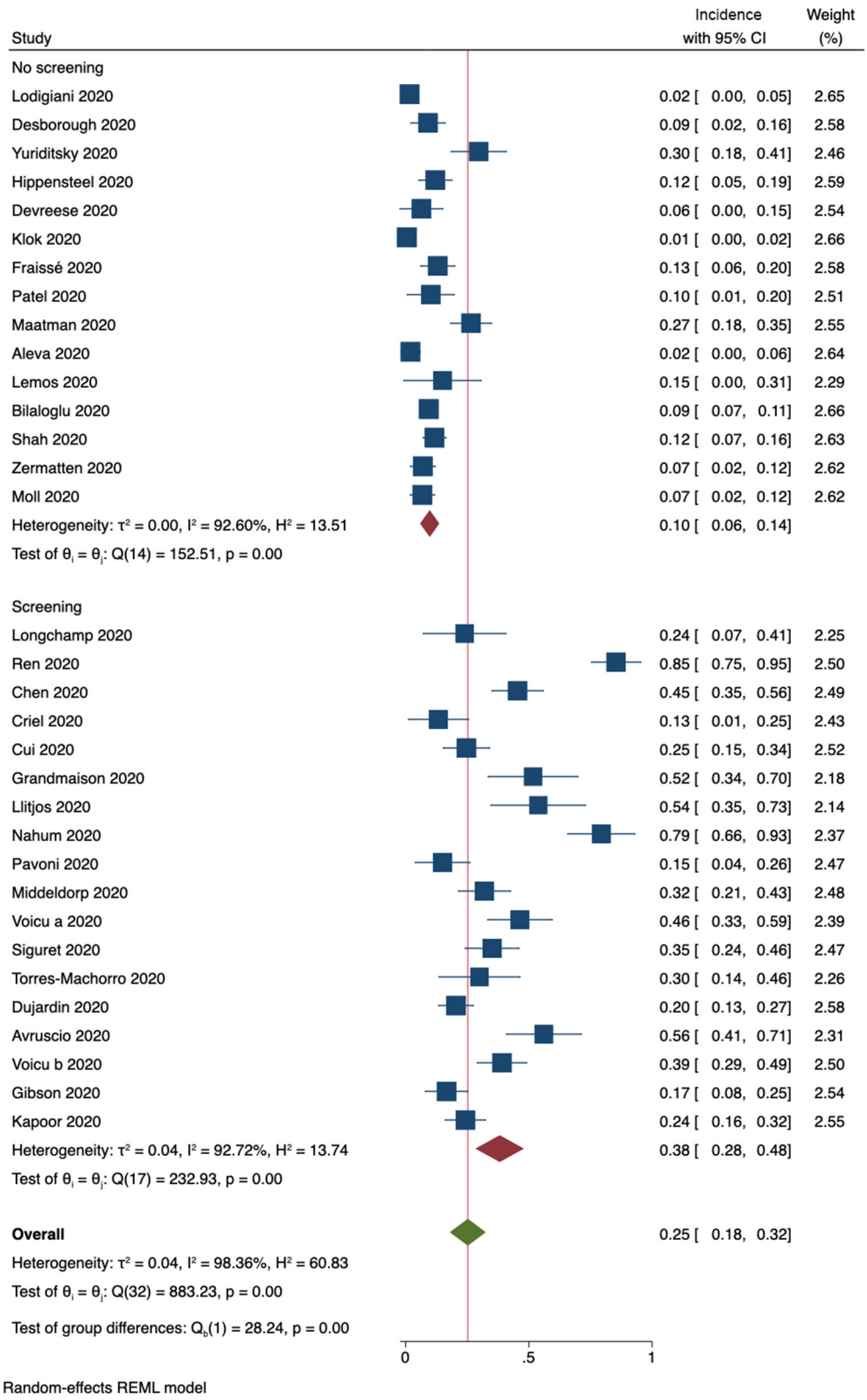

FIGURE 4 | Incidence of any form of DVT (33 studies). Forest plot shows subgroups according to whether routine ultrasound screening for DVT was performed.

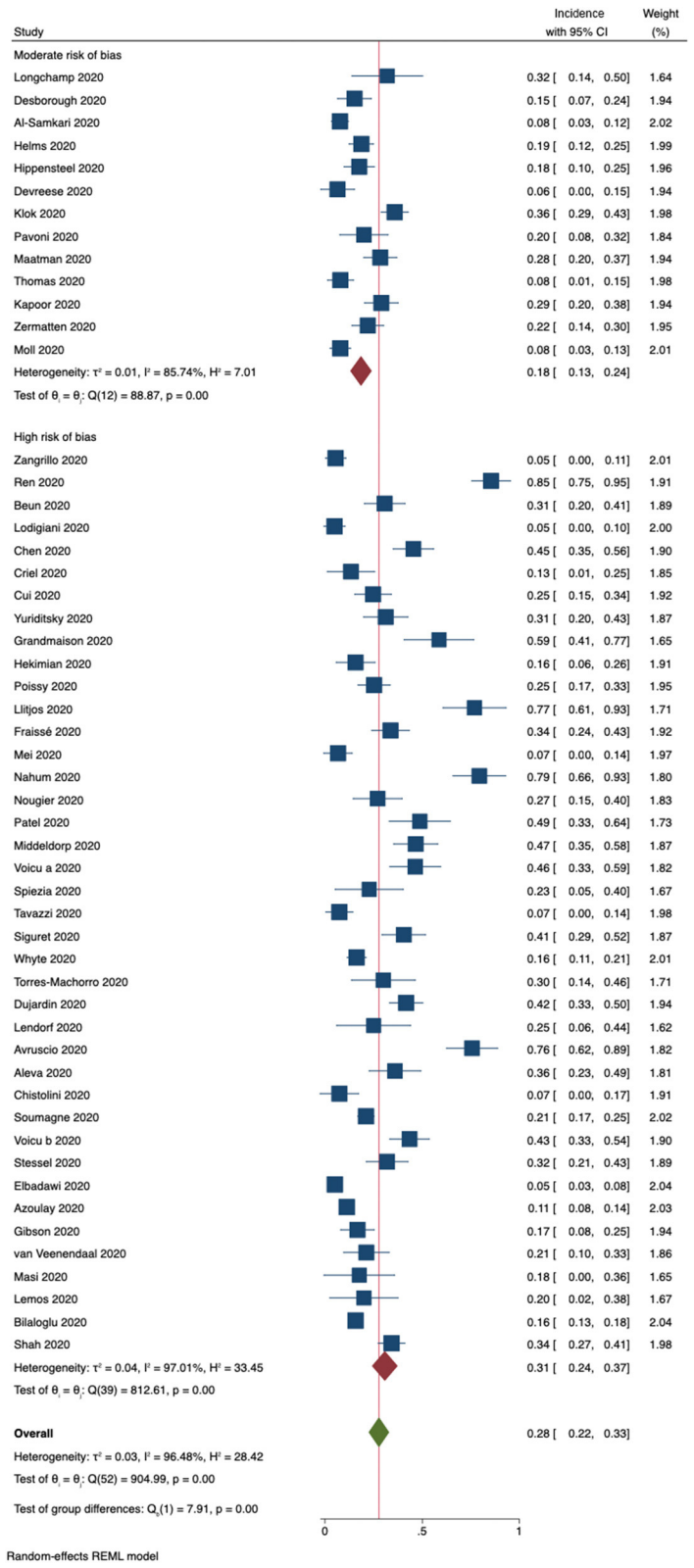

FIGURE 5 | Incidence of the composite outcome VTE (DVT + PE) for 53 included studies. Forest plot shows subgroups according to risk of bias.

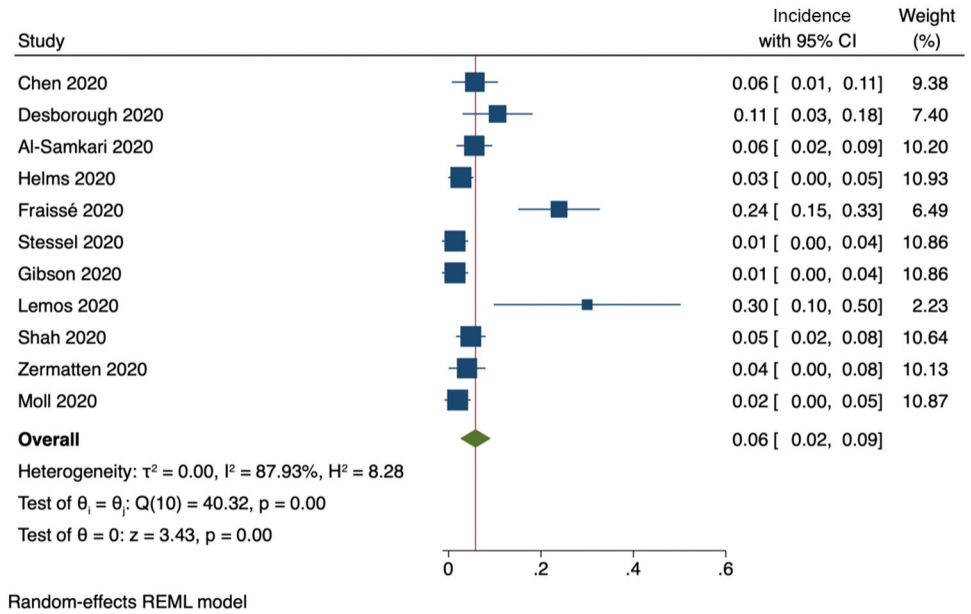

FIGURE 6 | Incidence of clinically relevant bleeding (11 studies).

sepsis. A recent study reported a VTE incidence rate of 37% in critically ill patients with sepsis, despite pharmacological thromboprophylaxis (80). Notably, routine ultrasound screening for DVT was used in this study. We found a comparable incidence rate of 44% for any form of VTE in studies that applied ultrasound screening for DVT. Another study reported a VTE incidence of 21% in a subgroup of critically ill patients with sepsis and acute respiratory distress syndrome, despite pharmacological thromboprophylaxis (81). In line with this, we found a pooled incidence of any form of VTE between 18 and 31%.

We observed a substantial degree of heterogeneity among the included studies. The reported VTE incidences in critically ill patients with COVID-19 vary widely, ranging from 0 to 85% (51, 52). A possible explanation and an important challenge when pooling the reported incidences is that different studies use distinct outcome definitions. For instance, only a subgroup of the studies reporting PE as an outcome parameter provided information to further characterize the form of PE. From a clinician's point of view, however, it is important to distinguish between a symptomatic patient with a central PE on one end of the spectrum and the incidental finding of a subsegmental PE in an asymptomatic patient on the other end of the spectrum. Along these lines, another possible explanation for the pronounced heterogeneity lies in the different diagnostic approaches. Current guidelines explicitly recommend against routine ultrasound screening for DVT in critically ill patients (10, 11). Interim guidance for the management of VTE in patients with COVID-19 adopted this recommendation (13). The underlying rationale for this recommendation is that routine screening might lead to the detection of asymptomatic, isolated distal DVT of questionable clinical relevance, which in turn might prompt the use of therapeutic anticoagulation in these patients and increase their bleeding risk. Nineteen of the 43 included studies that reported DVT incidence used ultrasound screening for the detection of DVT. Of note, both the DVT and overall VTE incidences were significantly higher in studies with screening than in studies without screening. Interestingly, when we pooled the incidences of proximal DVT, we found an incidence comparable with that in studies without ultrasound screening. A possible explanation may be the incidental detection of a high number of isolated distal DVT cases in screening studies. In line with this, a recent publication reported a 4-fold increase of isolated DVT with the use of ultrasound screening in COVID-19 patients in comparison with no screening (82). Similarly, the overall rate of any form of PE was positively correlated with the proportion of patients undergoing CT scans. Notably, this was not the case for the outcome parameter of non-subsegmental PE. Studies that provided details on the number of CT scans performed reported 514 CT scans in 1,433 patients. We hypothesize that the incidental detection of subsegmental PE of questionable clinical relevance in a substantial number of patients might have been caused by the high proportion of patients who underwent CT scans for other reasons. Recent publications highlight that the ideal diagnostic approach for the detection of VTE in patients with COVID-19 still remains unclear (17, 83).

Our work stands in contrast to previously published review articles that reported considerably higher incidence estimates for VTE in patients with COVID-19, particularly in those who are critically ill (84–91). For example, Shi et al. reported an estimated PE incidence of 19% for critically ill patients with COVID-19 (84). Similar incidence estimates (16–20%) were found by other meta-analyses as well (86, 87, 89). We report a significantly lower

incidence estimate (8%) for clinically relevant PE. Similarly, we found a lower pooled incidence rate for proximal DVT (14%) than that determined in previous meta-analyses, which reported incidence rates of up to 33% for any form of DVT (87). On the one hand, these discrepancies can be explained by the use of different outcome definitions, as we specifically focused on the clinical relevance of VTE. On the other hand, we also found lower overall incidences for any form of PE (13%) and DVT (22%) than earlier meta-analyses. This might be explained by the larger number of included studies in our work, with the notable inclusion of more recent studies. In contrast to an earlier review article, we did not observe a trend toward a lower VTE incidence over time (92). However, we did find that reported VTE incidence rates decreased as the study sample size increased. Especially among the first published studies, most contained small sample sizes and the majority of data originated from centers overwhelmed with an unexpectedly high number of severely ill patients with COVID-19.

Another relevant aspect that distinguishes the current work from previously published review articles is that we calculated pooled incidence estimates for bleeding episodes. Critically ill patients carry an inherent bleeding risk that needs to be weighed against the thromboembolic risk when administering pharmacological thromboprophylaxis. Importantly, the pooled incidence of 6% for clinically relevant bleeding episodes was not much lower than the rate of clinically relevant PE (8%).

Regarding the high heterogeneity of anticoagulant regimens reported in the included studies, it is noteworthy that in a corresponding subgroup-analysis, we did not observe differences in VTE incidence. However, it needs to be stressed that the current meta-analysis was not intended to detect differences in efficacy between distinct anticoagulation strategies. To date, only one prospective, randomized, controlled trial has compared different anticoagulation regimens in critically ill patients ($n = 20$) with COVID-19 (36). Therefore, it seems unlikely that a meta-analysis could shed light on this important question at this point. In line with this, a recently published Cochrane review concluded that there is currently insufficient evidence to determine the risks and benefits of anticoagulation in patients with COVID-19 (15).

Despite having a number of strengths, such as the focus on clinically relevant VTE, including data from different centers around the world and the considerable number of included patients, relevant limitations of our work need to be recognized. First, we observed substantial heterogeneity among studies that—apart from distinct outcome definitions—may have been caused by differences in study designs and settings. In particular, the absence of uniform diagnostic procedures to detect VTE needs to be borne in mind when interpreting the results of our study. Furthermore, we cannot exclude that the different included patient cohorts and different treatment strategies used in studies might have resulted in distinct VTE risks. Second, the inherent limitations of retrospective data reporting applied to the majority of the included studies. This is a likely explanation for our finding that all of the included studies had a moderate to high risk of bias. Third, particularly with regard to the earliest studies publication bias and small-study effects might have influenced our results.

In conclusion, the present study summarizes the globally available evidence on the incidence of clinically relevant VTE and bleeding events in critically ill patients with COVID-19. We calculated the incidences of PE and DVT separately and found significantly lower incidence rates than previous meta-analyses when focusing on clinically relevant event rates. Reported incidence rates varied to a high degree according to different diagnostic approaches. Considerable knowledge gaps remain, particularly with regard to the influence of different anticoagulant dosing regimens on VTE incidence. Future research is urgently needed to address this question by applying high-quality research standards, including the application of uniform outcome definitions, to guarantee comparability between studies. Meanwhile, the results of our study provide clinically important information with respect to an individual risk-benefit assessment of anticoagulant use in critically ill patients with COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JG was involved in the design of the study, literature review, data extraction, risk of bias assessment, statistical analysis, and drafted the manuscript. MW was involved in the design of the study, literature review, data extraction, and risk of bias assessment. MM was involved in the design of the study, data extraction, statistical analysis, and provided methodological support. HH was involved in the design of the study and the risk of bias assessment, provided methodological support, and undertook statistical analysis. HS was involved in the design of the study and literature review. EC was involved in the design of the study and performed the literature search. PK was involved in the design of the study and literature review. ES was involved in the design of the study, literature review, and data extraction. All authors contributed substantially to the writing of the manuscript, revised it, and approved it.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.647917/full#supplementary-material

Additional File 1 | Search strategy. Additional file 1 shows the detailed literature search performed in MEDLINE via OVID.

Additional File 2 | Supplementary Tables 1–3 show detailed study and patient characteristics and detailed quality assessments.

Additional File 3 | Supplementary Figures 1–7 show additional subgroup analyses and correlations performed for secondary outcome parameters.

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