The haematological presentations and mechanisms of thrombosis, the role of anti-coagulation prophylaxis, the management of coagulopathy as well as management of patients with COVID-19 disease and pre-existing bleeding disorders

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Summary

Severe COVID-19 infection obviously manifests hypercoagulability in its early stages and later could transform to hemorrhage. The DIC form in COVID-19 has unique features including frequent normal platelet counts, low anti-thrombin levels and hyperfibrinogenemia with progression to hyperfibrinolysis. A severe inflammatory response, originating in the alveoli, triggers a dysfunctional cascade of inflammatory thrombosis in the pulmonary vasculature, leading to a state of local coagulopathy. Numerous ongoing studies investigating the pathophysiology of the COVID-19 associated coagulopathy may provide mechanistic insights that can direct appropriate interventional strategies. Neutrophil /lymphocyte ratio and peak platelet/lymphocyte ratio may also have prognostic value in determining severe cases. During the disease course, longitudinal evaluation of lymphocyte count dynamics and inflammatory indices, including LDH, CRP and IL-6 may help to identify cases with dismal prognosis and prompt intervention in order to improve outcomes. Elevated D-Dimer levels are consistently reported, whereas their gradual increase during disease course is particularly associated with disease worsening. Other coagulation abnormalities such as PT and aPTT prolongation, fibrin degradation products increase, with severe thrombocytopenia lead to life-threatening disseminated intravascular coagulation (DIC),

which necessitates continuous vigilance and prompt intervention. The initial coagulopathy of COVID-19 presents with prominent elevation of D-dimer and fibrin/ fibrinogen degradation products, whereas abnormalities in prothrombin time, partial thromboplastin time, and platelet counts are relatively uncommon in initial presentations. COVID19–associated coagulopathy should be managed as it would be for any critically ill patient, following the established practice of using thromboembolic prophylaxis for critically ill hospitalized patients, and standard supportive care measures for those with sepsis-induced coagulopathy or DIC. D-dimer level has greater advantage in predicting mortality and clotting status including pulmonary embolism and deep venous thrombosis. Although D-dimer, sepsis physiology, and consumptive coagulopathy are indicators of mortality, several studies have shown that hematological parameters are markers of disease severity and suggest that they mediate disease progression.

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Abbreviation

FVIII: Factor VIII
IL: Interleukin
NET: Neutrophil extracellular trap
TF: Tissue factor
TNF: Tumor necrosis factor
VWF: von Willebrand factor
PT: prothrombin time
VTE: Venous thromboembolism
ARDS: Acute Respiratory Distress Syndrome
ACE2: angiotensin-converting enzyme 2
MERS: Middle East Respiratory Syndrome
Aptt: activated partial thromboplastin time
SIC: sepsis induced coagulopathy
TMA: thrombotic microangiopathy
GMCSF: granulocyte-macrophage colony-stimulating factor
DIC: disseminated intravascular coagulopathy
MAS: macrophage-activation syndrome
NETs: Neutrophil Extracellular Traps
ANC: Absolute Neutrophil Count
TTE: Transthoracic echo
ICU: intensive care unit
ECMO: extracorporeal membrane oxygenation
CRRT: continuous renal replacement therapy
LMWH: low molecular weight heparin
UFH: unfractionated heparin
DOAC: direct oral anticoagulant
UFH: unfractionated heparin

1. Introduction

The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China at the end of 2019 and is now a pandemic (1). SARS–CoV–2 is a member of the subgenus Sarbecovirus of the genus Betacoronavirus in the family Coronaviridae (2-4). The family, with a large genome of 26–32 kilobases, belongs to a group of enveloped positive–sense single–stranded ribonucleic acid (RNA) viruses, referred to as (+)ssRNA viruses (5),(6). Respiratory infections such as influenza and other flu-like upper respiratory viral diseases, bacterial pneumonia, and COPD exacerbation all commonly present during winter time, coinciding with the emergence of the pandemic. In addition, cardiovascular morbidity such as heart failure and venous thromboembolism (VTE) may lead to respiratory distress symptoms resembling COVID-19. The SARS-CoV-2 infection may manifest as mild, moderate, or severe on clinical presentation. The common manifestations reported in patients with COVID-19 are fever (most common symptom), dry cough, dyspnoea, sputum production, fatigue, myalgia, arthralgia, sore throat, headache and gastrointestinal symptoms such as nausea, vomiting and diarrhoea. Cytokine storm in the form of acute respiratory distress syndrome (ARDS), respiratory failure, sepsis and septic shock are among the severe presentations described. This pandemic is attributed to human-to-human transmission (within 1 m) caused by respiratory droplets such as coughing and sneezing, and by indirect contact with contaminated surfaces or objects including thermometers and stethoscopes (7).

Coagulopathy, in the form of venous and arterial thromboembolism, is emerging as one of the most severe sequela of the disease, and has been prognostic of poorer outcomes (8-11). Reports of high incidence of thrombosis despite prophylactic and therapeutic dose anticoagulation raise question about a pathophysiology unique to COVID-19 (12),(13). Proposed hypotheses include a severely heightened inflammatory response that leads to thromboinflammation, through mechanisms such as cytokine storm, complement activation, and endotheliitis (9),(10),(14),(15).

Note, SARS-CoV-2 is approximately 80% similar to SARS-CoV, and invades host human cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor (3). COVID-19 is primarily manifested as a respiratory tract infection, emerging data indicate that it should be regarded as a systemic disease involving multiple systems, including cardiovascular, respiratory, gastrointestinal, neurological, hematopoietic and immune system (16-18). Mortality rates of COVID-19 are lower than SARS and Middle East Respiratory Syndrome (MERS) (19); however, COVID-19 is more lethal than seasonal flu.

Severe illness is designated when the patients have fever or suspected respiratory infection, plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or pulse oximeter oxygen saturation ≤ 93% on room air (19). Critical illness is defined as patients with acute respiratory distress syndrome or sepsis with acute organ dysfunction (19). COVID-19 is associated with marked abnormalities in markers of hypercoagulability, including elevated levels of D-dimer, fibrinogen, and factor VIII, a shortened activated partial thromboplastin time (aPTT) and an elevated sepsis induced coagulopathy (SIC) score (16). Specific objectives are: 1) to provide an approach to the diagnosis of VTE; 2) to provide guidance on thromboprophylaxis strategies in ICU and non-ICU settings, including the duration of prophylaxis; and 3) to provide guidance on the treatment of VTE.

Yuki et al.,(20), classified the disease manifestation into five separate categories, namely: (a) asymptomatic; (b) disease with mild symptoms (including fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing, nausea, vomiting, abdominal pain and diarrhea); (c) moderate disease (pneumonia with frequent fever, cough without obvious hypoxemia and computed tomography (CT) of the chest showing lesions); (d) severe disease (pneumonia with hypoxemia and peripheral oxygen saturation, SpO2 < 92%); and (e) critical disease (associated with ARD, shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction and acute kidney injury). More recently, three main stages of the disease have been defined: stage I (mild symptoms observed) and stage II (pulmonary involvement detected) both lasting 5–7 days, with stage II being further divided into two substages, II A (no hypoxia) and II B (with hypoxia). The most severe stage, stage III (systemic inflammation) is attained by approximately 10–15% of patients (21). Although diagnosis of coronavirus disease 2019 (COVID–19) is challenging in the early stages due to non–obvious manifestations, hematological signs and symptoms provide clues to aid diagnosis.

Retrospective studies have identified clinical parameters that predict poor prognosis. In addition to markers of coagulopathy such as Ddimer other hematologic parameters have been studied,(10),(11),(9, 22-24). Neutrophil count, lymphocyte count, neutrophil /lymphocyte ratio, and platelet count correlate with disease severity (9),(25-27). Several recent studies suggest a hypercoagulable state in patients presenting with COVID-19. Laboratory findings show high CRP, leukopenia, lymphocytopenia, mild thrombocytopenia, prolonged PT, high D-dimers, and high fibrinogen levels early in the disease course, which may be complicated by low fibrinogen later on in severe cases (11, 22, 28). There is experimental evidence that human transmembrane angiotensin–converting enzyme 2 (ACE2) serves as a receptor for SARS–CoV–2 (29-32).Briefly, the receptor–binding spike (S) glycoproteins, located on the surface of the virus, attach to the host cell through ACE2 and, consequently, the viral RNA may enter the host cell and replicate (30-33)

In this article discuss Thrombocytopenia in patients with COVID-19, Inflammation and coagulation, The hypercoagulable state with COVID-19, Endotheliopathy and COVID-19, The possible mechanism of thrombocytopenia in COVID-19, Inflammatory thrombosis, Pathogenesis of thrombosis and DIC associated with COVID-19 infection, Impact of blood count abnormalities, Risk of VTE in COVID-19,Incidence of VTE in Patients with COVID-19, Thrombotic manifestations associated with COVID-19 infection, Difficulties in the Diagnosis of VTE in Patients with COVID-19, Full blood count and biochemistry findings, HEMATOLOGIC PAR AMETERS OF PATIENTS WITH COVID-19 INFECTION, VTE Prophylaxis in non-ICU and in ICU Hospitalized COVID-19 Patients, Hematologic parameters to predict COVID-19 prognosis, Anti-coagulation therapy, Management of thrombotic risk

and bleeding episodes,Treatment of VTE Patients with COVID-19 Duration of Thromboprophylaxis in Hospitalized COVID-19 Patients and VTE Treatment in Hospitalized COVID-19 Patients

2. Thrombocytopenia in patients with COVID-19

The most common symptoms seen in COVID-19 patients are fever, fatigue, and dry cough, and dyspnea gradually develops. Some patients have mild symptoms at disease onset and may not present with apparent fever. Uncommon symptoms include abdominal pain, headache, palpitations, and chest pain. Hematological changes are common in patients with COVID-19, which include reduced lymphocyte count and platelet count but normal white blood cell count. Prolonged activated partial thromboplastin time, 26% had elevated D-dimer levels, and most patients had normal prothrombin time (PT) (8). Of seven patients in the University of Hong Kong-Shenzhen Hospital (Shenzhen, Guangdong province, China), two had thrombocytopenia, and two had elevated D-dimer levels (34). A study involving 1099 patients from 31 provinces/direct-controlled municipalities in China showed that 82.1% of patients had lymphopenia, 36.2% had thrombocytopenia, and 33.7% had leukopenia (35). These laboratory marker abnormalities were more significant in severe cases (35). In 13 patients from 3 hospitals in Beijing, 72.5% developed thrombocytopenia (36). Statistics from 41 patients in a designated hospital in Wuhan showed that 5% of patients had thrombocytopenia on admission (37). In most cases, the platelet count did not decrease to a level at which bleeding occurs.

3. Inflammation and coagulation

Activation of host defense systems results in subsequent activation of coagulation and thrombin generation as critical communication components among humoral and cellular amplification pathways, a term called thromboinflammation or immunothrombosis (38-40). In patients with SIC, the importance of the evolution from adaptive hemostasis to pathologically induced DIC with multiorgan failure continues to be evaluated.

Coagulation is activated by the inflammatory response through several procoagulant pathways. Polyphosphates, derived from microorganisms, activate platelets, mast cells, and factor XII (FXII) in the contact pathway of coagulation, and exhibit other downstream roles in amplifying the procoagulant response of the intrinsic coagulation pathway (41). Complement pathways also contribute to activation of coagulation factors (42). Although neutrophil extracellular traps are present in thrombi, the individual neutrophil extracellular trap components of cell-free DNA and histones activate the contact pathway and enhance other prothrombotic pathways resulting in thrombin generation (43),(44).

Pathogen-associated molecular mechanisms are important aspects of the complex interactions between the immune response and coagulation and in sepsis (43),(45). The inflammatory effects of cytokines also result in activated vascular endothelial cells and endothelial injury with resultant prothrombotic properties (43),(46).

Critically ill patients at high risk of mortality may benefit from strategies to inhibit these responses, but the success of interventions may depend on the time course and evolution of the infection. Circulating serine protease inhibitors including antithrombin, C1 esterase inhibitor, and protein C are decreased in the setting of the inflammatory response to infection (47). Fibrinolytic shutdown that also occurs in sepsis is characterized by increased PAI-1 activity, resulting in low D-dimers (48),(49). Vascular endothelial

injury not only causes further thrombocytopenia and reduction of natural anticoagulants, but also hemostatic activation as the phenotypic expression of thrombotic DIC.

Subsequent reductions in coagulation factors associated with increased fibrinolysis that can occur during infections and sepsis are considered the fibrinolytic phase of DIC (50),(39). As evidenced by the data from Tang et al, SIC and overt DIC occur in patients in later stages of COVID-19 infection, while still hospitalized, often with septic physiology and multi-organ failure (11),(51).

Significant inflammation is present in patients with SARS-CoV-2 infection, based on elevated levels of IL-6, increased C-reactive protein and erythrocyte sedimentation rate, and elevated fibrinogen at presentation (52). Given the tropism of the virus for ACE2 receptors, endothelial cell activation and damage with resultant disruption of the natural antithrombotic state is likely.

A report of COVID-19 patients in Wuhan measured proinflammatory cytokines and found elevated plasma concentrations that were higher in ICU patients than in non-ICU patients (10). This inflammation associated with COVID-19 and subsequent activation of coagulation is the probable cause for the elevated D-dimer levels, as increased levels have been associated with many conditions other than thromboembolism, with infection an important etiology (47),(53),(54).

SARS-CoV-2, patients appear with systemic inflammatory response syndrome or cytokine storm, which may explain more dramatic changes in coagulation tests, including significantly elevated D-dimer, especially as the disease progresses (18). As Tang et al demonstrated, fibrinogen levels in all patients were elevated on admission (11). Ranucci et al reported on 16 COVID-19 patients with acute respiratory distress syndrome (ARDS) requiring mechanical ventilation who had fibrinogen, D-dimer, and IL-6 levels measured. An important finding from this report was that increased IL-6 levels correlated with increased fibrinogen levels, demonstrating and confirming the link between inflammation and procoagulant changes; all patients had elevated IL-6 levels on admission (55).

Elevated D-dimer levels at admission or increasing D-dimer over time are both associated with increased mortality with COVID19. Patients who develop septic physiology and septic shock are at increased risk of death, as are those who develop DIC even if it occurs in the absence of sepsis. The mechanisms that activate coagulation in SARS-CoV-2 infection are appear to be linked to inflammatory responses rather than specific properties of the virus (56).

4. The hypercoagulable state with COVID-19

Previous outbreaks of coronaviruses, including SARS-CoV-1 and Middle-Eastern respiratory syndrome (MERS-CoV) have been associated with increased risk of thrombosis (57). Similarly, the novel SARS-CoV-2 appears to generate a profoundly prothrombotic milieu as evidenced by a surge in global reports of arterial, venous and catheterrelated thrombosis (8),(12),(58).

4.1. Venous thromboembolism

Pulmonary embolism is the most common thrombotic manifestation of COVID-19 (59). One of the first substantial datasets on risk of venous thromboembolism (VTE) in critically ill patients with COVID-19, reported a VTE incidence of 25% (22). In a larger study in the Netherlands, 184 ICU patients with COVID-19 who were all on at least standard thromboprophylaxis had a 27% cumulative incidence of

VTE, with pulmonary embolism (PE) being most frequent (81%) (12). Middeldorp et al. reported a higher incidence of thrombotic complications in their ICU patient population (7-day and 14-day cumulative incidence of 25% and 48% respectively) compared to the patients admitted on the wards (58). Another data set from France that included 150 patients with COVID-19 associated acute respiratory distress syndrome (ARDS) showed a VTE rate of 18%, with PE being most common. When compared to a historical prospective cohort of non-COVID-19 ARDS after matching, patients with COVID-19 ARDS demonstrated a significantly higher rate of thrombotic events, mainly PEs (11.7% vs 2.1%, OR 6.2, p = 0.008) (60).

4.2. Arterial thrombosis

4.2.1. Myocardial infarction

MI has not been commonly reported However, In the Italian study by Lodigiani et al which included 388 patients with COVID-19, the incidence of MI or acute coronary syndromes was 1.1% (61). Troponin levels have been noted to be significantly higher in the non-survivors, and may provide prognostic value (8),(62). While there are many explanations for elevated troponins (renal injury, myocarditis), ischemic injury as a result of plaque rupture and consequent infarction or secondary to demand ischemia has been reported, and is postulated as another cause of myocardial injury (15).

4.2.2. Stroke

Oxley et al have reported an alarming seven-fold increase in large vessel strokes in the < 50-year-old age group in New York City, New York (5 patients in a 2-week period during COVID-19 pandemic compared with 0.7 patients pre-COVID)(63). Another case series reports three patients with COVID-19 presenting with strokes and limb ischemia(58). Clinical presentation with ischemic strokes was also noted by Klok et al (3.7%) and Lodigiani et al (2.5%)(12), (61).

4.2.3. Microvascular thrombosis

Several clinical reports have demonstrated evidence of thrombotic microangiopathy (TMA) in patients with COVID-19, most notably in lung autopsies(64-66). In a study by Menter et al, five out of eleven patients showed evidence of microthrombi in lung autopsies (65), and another case series by Ackermann et al. showed widespread thrombosis with microangiopathy in the lung autopsies of seven COVID-19 patients (66). The authors compared these findings to those of severe influenza patients, and found that alveolar microthrombi were 9 times more prevalent in COVID-19 patients ($p < 0.001$) (66). Interestingly, one report from China described the presence of extensive microvascular thrombosis in extrapulmonary organs where coronavirus was not detected, suggesting that a mechanism beyond viral infection is operative (67). Tian et al provided evidence of microthrombi in pathological samples obtained from two asymptomatic patients who underwent lobectomies for lung adenocarcinoma, and were subsequently found to be positive for COVID-19 (68). Pathological examinations revealed patchy inflammatory cellular infiltrate with focal areas of fibrin deposit, suggesting that a local hypercoagulable state in the

pulmonary tissue may be an early occurrence. Furthermore, TMA may contribute to findings of unusual sites and presentations of thrombosis that have been described in several clinical reports. COVID-19 induced chilblains eruption has been reported, where histopathology revealed microangiopathy thought to be induced by a robust interferon response to the virus(69). Chilblain-like lesions have also been reported in otherwise asymptomatic adolescents (70). Transient livedo reticularis has been reported in two patients and is believed to be secondary to coagulopathy(71). Several reports have mentioned cases with bowel ischemia(15). limb or acral ischemia (58),(72), and cutaneous ischemia(58).

5. Endotheliopathy and COVID-19

Consistent with vascular endothelial dysfunction with SIC, an endotheliopathy appears to contribute to the pathophysiology of microcirculatory changes in SARS-CoV-2 infections (46),(73). The receptor for viral adhesion is an ACE2 receptor on endothelial cells,(74), with viral replication causing inflammatory cell infiltration, endothelial cell apoptosis, and microvascular prothrombotic effects (15). Recent reports demonstrate viral inclusions within endothelial cells and sequestered mononuclear and poly-morphonuclear cellular infiltration, with evidence of endothelial apoptosis in the postmortem of SARS-CoV-2 infection (15). From a clinical perspective, in addition to the systemic hypercoagulability and potential for thromboembolic complications, the described microvascular endothelial injury with microcirculatory clot formation noted in postmortem evaluation is consistent with a thrombotic microangiopathy that may occur in patients (15). The endotheliopathy may also explain reports of cerebrovascular complications in younger patients, myocardial ischemia, and increasing reports of both micro- and macrocirculatory thromboembolic complications (15, 47, 50).

6. The possible mechanism of thrombocytopenia in COVID-19

6.1. SARS-CoV-2 may reduce platelet production

Coronaviruses are able to infect bone marrow cells, resulting in abnormal hematopoiesis (75). SARS-CoV-2 and human SARS-CoV have 82% nucleotide homology (76). Because SARS-CoVand HCoV-229E have identical antigen characteristics, it is speculated that SARS-CoV-2 and HCoV-229E antigens have some similarity. Human aminopeptidase N (CD13) is a metalloprotease that is present on the cell surfaces of epithelial cells in the intestine, kidneys, and lungs and is a receptor for HCoV-229E (77). CD13 is a marker of granulocytes and monocytes and is ubiquitous in respiratory tract epithelial cells, smooth muscle cells, fibroblasts, epithelial cells in the kidneys and small intestine, activated endothelial cells, lymphocytes, and platelets. HCoV-229E enters bone marrow cells and platelets through CD13 receptors and induces growth inhibition and apoptosis in the bone marrow, leading to aberrant hematopoiesis and thrombocytopenia (77). Thrombocytopenia caused by SARS-CoV-2 infection is similar to that caused by SARS-CoV and HCoV-229E infection. Based on this phenomenon, it is speculated that SARS-CoV-2 similarly inhibits hematopoiesis in the bone marrow through certain receptors to cause decreased primary platelet formation and lead to thrombocytopenia Secondary hemophagocytic lymphohistiocytosis (sHLH) is caused by excessive proliferation and activation of mononuclear macrophage system, in which a large number of inflammatory cytokines are released and a large number of blood cells are swallowed. SARS-CoV-2 infection has a rapid response with high mortality, and its basic features include persistent fever, hyperferremia, cytopenia, and lung involvement. In the retrospective analysis of 150 patients with COVID-19 in Wuhan, China, it was found that elevated

ferritin was one of the predictors of death(18). After analyzing the blood samples of 33 severe and critical type ill COVID-19 patients, Wei Haiming's team found that after novel coronavirus infection, T cells were over activated to produce granulocyte-macrophage colony-stimulating factor (GMCSF) and interleukin-6 (IL-6). GM-CSF stimulated CD14+ CD16+, inflammatory mononuclear macrophages to produce more interleukin-6 (IL-6), and other inflammatory factors, thus forming an inflammatory storm and causing immune damage to the lungs and other organs(78).

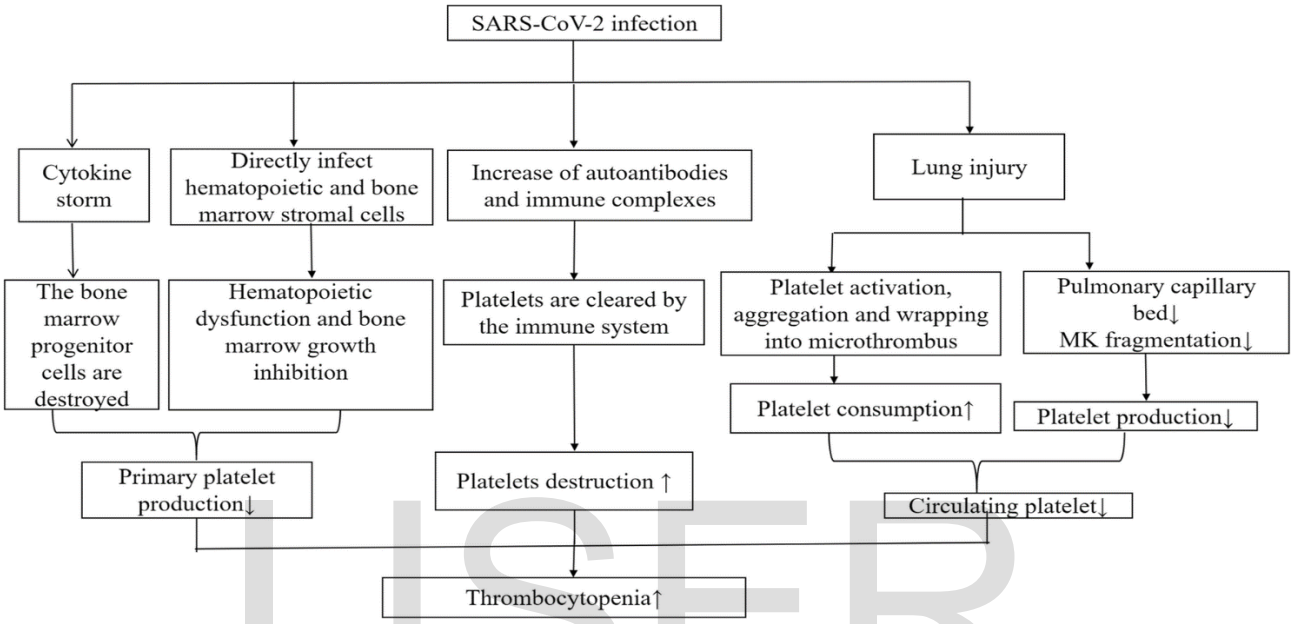

Fig. 1 The possible mechanisms of thrombocytopenia in COVID-19 patients.

Studies have shown(18), that the cytokine spectrum similar to sHLH is related to the severity of COVID-19 disease. It is speculated that after the cytokine storm, the hematopoietic progenitor cells in bone marrow of patients with pneumonia infected by novel coronavirus were destroyed, the primary production of platelets decreased, and at the same time, too many blood cells were swallowed, which led to the decrease of peripheral blood platelet count.

Evidence(79), has shown that a large number of megakaryocytes dynamically release platelets during pulmonary circulation. Persistent hypertension and oxygen toxicity exacerbate lung injury, resulting in consolidation changes such as fibrosis. Damaged pulmonary capillary beds cause the process of megakaryocyte rupture and platelet release to be blocked, which affects the release of platelets into the pulmonary circulation and indirectly leads to reduced platelet synthesis in the systemic circulation.

7. Pathophysiology of COVID-19 coagulopathy: inflammatory thrombosis

In addition to bedside evidence for a hypercoagulable state in COVID-19, laboratory tests have also been consistent with a prothrombotic milieu such as increased D-dimer, fibrinogen, factor VIII (FVIII), von Willebrand factor (vWF), decreased antithrombin, and TEG results (79). While critical illness is known to cause a hypercoagulable state due to immobilization, mechanical ventilation, central venous access devices, and nutritional deficiencies, COVID-19 appears to cause a hypercoagulable state through

mechanisms unique to SARS-CoV-2 and centers around the cross-talk between thrombosis and inflammation(80),(55).

COVID-19 causes a profoundly pro-inflammatory state, as evident from multiple reports of high C-reactive protein, lactate dehydrogenase, ferritin, interleukin-6 and D-dimer levels(81). IL-6 and fibrinogen levels are shown to correlate with each other in COVID-19 patients, providing credence to the idea of inflammatory thrombosis (55). Researchers presently believe that the inciting event sparking the cycle of inflammation and thrombosis originates in the pulmonary alveoli, where SARS-CoV-2 enters the alveolar epithelium through the ACE2 receptor. Consequently, a severe inflammatory response is initiated that sets the stage for thrombosis through several mechanisms.

7.1. Localized intravascular coagulopathy

A report by Tang et al. described a high rate (71.4%) of COVID-19 patients meeting ISTH disseminated intravascular coagulopathy (DIC) criteria(11). However, clear evidence of overt clinical DIC in COVID-19 is lacking thus far. It is possible that the laboratory abnormalities noted are a reflection of a localized coagulopathy in the pulmonary vasculature, resulting from severe alveolar inflammation (23),(82). Progression of this process to the systemic circulation may explain microthrombotic complications and ensuing multi-organ failure(83). The initial viral damage occurring in the alveoli generates inflammation and local microvascular pulmonary thrombosis. This is followed by more generalized endothelial dysfunction and thrombo-inflammation in the microvasculature of the brain, kidneys and other organs leading to a hyper-coagulable state and multiple organ failure.

7.2. Inflammatory cytokines

Excessive cytokine release is postulated to cause the severe illness noted in younger patients without pre-existing conditions. Higher serum levels of several inflammatory cytokines and chemokines have been associated with severe illness and death in multiple studies (84-87). The cytokine profiles in patients with severe COVID-19 show increased production of IL-6, IL-7, TNF, and inflammatory chemokines such as CCL2, CCL3, and soluble IL-2 receptor, a profile similar to that seen in cytokine release syndromes, such as macrophage activation syndrome (88). Excessive cytokine release contributes to thrombosis through multiple mechanisms, including activation of monocytes, neutrophils, and the endothelium, all of which generates a prothrombotic state.

7.3. Endothelial activation & dysfunction

Varga and colleagues first reported endothelial dysfunction in multiple vascular beds on post mortem specimens obtained from three patients (15). In case series of 7 patients by Ackerman et al, lung autopsies of COVID-19 patients showed severe endothelial injury with presence of intracellular virus, as well as widespread thrombosis with microangiopathy(66). Furthermore, significantly elevated levels of VWF and FVIII in COVID-19 patients are suggestive of endothelial activation in these patients (60),(89). Endothelial activation or dysfunction with COVID-19 may occur through multiple mechanisms. This includes inflammatory cytokines generated in the pulmonary interstitium, the activation of the complement components in blood, or possibly, as a direct result of SARS-CoV-2 infection of endothelial cells through the ACE2 receptor(90). Endotheliitis, in turn, is a major forerunner to thrombosis. The observation that male sex, obesity, hypertension, and diabetes are poor prognostic factors for severe

disease with COVID-19 further supports this theory due to the presence of endothelial dysregulation at baseline in these patients (15),(60), (58), (89),(90).

7.4. Mononuclear phagocytes (MNPs)

Monocytes and macrophages are theorized to play a crucial role in the inflammation and thrombosis seen in COVID-19. Liao et al demonstrated that MNPs account for 80% of the total broncho-alveolar fluid from patients with severe COVID-19 illness, compared to 60% and 40% in mild cases and healthy controls, respectively(91). Furthermore, the composition of the cells was characterized by an abundance of inflammatory monocyte-derived macrophages in patients with severe disease. Broncho-alveolar fluid in severe patients is enriched with chemokines potently recruit monocytes(92). COVID-19 patients requiring ICU hospitalization were noted to have a significant expansion of CD14+, CD16+ monocyte populations producing IL-6 in peripheral blood(88),(93-95).

In another study, circulating monocytes were shown to have a sustained production of TNF-α and IL-6, a pattern that differs from bacterial or influenza sepsis(96). Similarly, post-mortem analyses of COVID-19 positive patients revealed that lymphoid tissue macrophages infected with SARS-CoV-2 viral particles expressed IL-6. Further, the presence of IL-6+ macrophages was associated with severe depletion of lymphocytes from lymphoid tissue(97). These findings suggest that COVID-19 is associated with a clinical and laboratory picture similar to that of macrophage-activation syndrome (MAS).

However accumulating data suggests a role for MNPs in the generation of severe illness, including possibly the prothrombotic sequelae. The triggers the coagulation cascade resulting in production of thrombin which in turn leads to thrombus generation, platelet activation, and amplification of pro-inflammatory pathways, primarily through PAR signaling (98).

7.5. Neutrophil extracellular traps (NETs)

NETs are implicated to portend pathogenicity in a wide variety of disorders including influenza-associated ARDS (99), and thrombo-inflammation(100),(101). Yu et al report elevated levels of serum NETs in hospitalized COVID-19 positive patients based on a finding of elevated cell-free DNA, myeloperoxidase-DNA and citrullinated histones in 50 patients with COVID-19. This was especially noted in hospitalized and mechanically ventilated patients(102). Moreover, they showed that sera obtained from these patients stimulated NET generation in control neutrophils. Taken together with literature linking NETs to pulmonary diseases and thrombo-inflammation, these data begin to implicate NETs as causative in organ damage, widespread thrombosis and mortality that is noted in COVID-19 infection. Finally, a recent manuscript by Barnes et al describes an abundance of neutrophil infiltration in pulmonary capillaries of three patients who succumbed to COVID-19 and suggests that aberrant activation of neutrophils and NET generation may underlie the cytokine storm and severe disease outcomes noted in this disease(103).

7.6. Complement-mediated microangiopathy

Previous studies in animal models provide evidence for complement activation in serum and pulmonary tissue(104),(105). A growing body of evidence suggests a major role for dysregulated complement activation in severe COVID-19(106). Several reports of post-mortem examinations have demonstrated

evidence of TMA, including hyaline thrombi in the small vessels of the lungs and other organs(107),(108). One of those reports demonstrated lung and skin biopsy findings revealing a pauci-inflammatory thrombogenic vasculopathy with complement deposit. Researchers in China observed complement hyper-activation in COVID 19 patients, as well as significantly increased plasma C5a levels in severe cases(109). Dysregulated complement system activation may be a major contributor to cytokine storm, particularly through the pro-inflammatory effects of anaphylatoxins C3a and C5a(110). [These effects are likely to become more detrimental in patients with a genetic predisposition for decreased complement regulation, and may contributed to findings of TMA and subsequent organ dysfunction.

7.7. Dysregulated renin angiotensin system (RAS)

Although dysfunction of RAS is known to play a significant role in ARDS in general (111),(112), this system is specifically important in COVID-19 infections for several reasons. The SARS-CoV2 uses its Spike (S) protein and fuses with the enzyme Angiotensin-Converting Enzyme 2 (ACE2) located on the cell membrane of human cells to gain entry into cells. ACE2 is homologous to ACE, which cleaves angiotensin I (ANGI) to generate ANGII. ANGII binds to the Angiotensin Type I Receptor (AT1R) that leads to vasoconstriction and an increase in blood pressure. The inactivation of ANGII by ACE2 results in vasodilation.

Conversely, ANGII also negatively regulates ACE2(113), which is located on lung alveolar epithelial cells, renal tubular epithelial cells, enterocytes of the small intestine, endothelial cells, cardiomyocytes, fibroblasts and pericytes in the heart. SARS-CoV-2 has a high affinity for ACE2, and binding of the SARS-CoV-2 results in loss of ACE2 due to internalization of the virus and ACE2 shedding. This decrease in ACE2 leads to decreased degradation of ANGII resulting in excess ANGII binding to AT1R and increase lung injury(113). Finally, studies suggest that ANGII binding to AT1R may stimulate IL-6 release, further contributing to the cytokine storm syndrome that is typical of severe COVID-19 infection. Supporting this hypothesis is the evidence showing increased risk of severe disease in patients infected with SARS-CoV or influenza H7N5 that had higher ANGII levels (114),(115). Further, in recent studies on COVID-19 positive patients, viral load and lung injury directly correlated with plasma ANGII levels(116). In addition,COVID-19 appears to generate worse outcomes in patients with hypertension, cardiovascular disease and diabetes, all of which are associated with reduced baseline levels of ACE2 expression suggesting imbalance in ACE/ACE2 levels (117). It has been shown that ANGII induces TF and plasminogen activator inhibitor 1 (PAI-1) expression by endothelial cells via AT1R, leading to a hypercoagulable state(118),(119). Presently, rapid investigation into the molecular mechanisms involved is essential in order to gain a better understanding of the pathophysiology of the disease and to direct appropriate, timely therapeutic interventions.

8. Pathogenesis of thrombosis and DIC associated with COVID-19 infection

Critically ill patients fulfilled all three criteria of Virchow's triad including reduced venous flow due to immobility, endothelial injury by direct invasion of the COVID-19 virus or central venous catheter use and prothrombotic changes (120). Hyperfibrinogenemia is the major risk of thrombosis. Even though COVID-19 induced DIC, the fibrinogen in early stage still remained high indicating low consumption of fibrinogen, the hallmark sign in COVID-19. Elevated factor VIII is also caused by the inflammatory process and revealed many causes of infection including COVID-19. Increased circulating prothrombotic microparticles as a result of platelet and monocytes destruction, which is well known among septic

patients, may also occur in COVID-19 cases. Activated neutrophils released Neutrophil Extracellular Traps (NETs), promoting hypercoagulability. Another elevated parameter is von Willebrand factor will also raise the concern to cause other events. The pathophysiology of DIC is complex. The activation of vascular endothelium, platelets and leukocytes can induce the release of cytokines resulting in systemic thrombin dysregulation. The deposition of fibrin will subsequently thrombose and damage tissues, especially the microvascular tissues in the lung.

The exaggeration will also inhibit fibrinolysis and impair anticoagulant mechanisms (121). Interestingly, at the late stage of COVID-19 infection, the level of fibrin-related markers (D-dimer and FDP) is markedly elevated. Therefore, secondary hyperfibrinolysis occurs in this setting (11) and so with coagulopathy would replace the hypercoagulable state due to acute inflammatory the response process (122). Different clinical manifestations of DIC, emerging in the late stage of COVID-19 infection, from others causes of DIC are described below.

1. Some laboratory data especially platelet count may not show compatibility with DIC even when a full-blown status is encountered. Because organ dysfunction is mainly limited in the lungs, this condition induces an increase in thrombopoietin level following pulmonary inflammation. In one study, only 21.6% of severe cases met the sepsis induced coagulopathy (SIC) criteria, consisting of platelet count, PT-INR, SOFA score, meaning cases that fulfilled the criteria for treatment are limited (51).

2. The ISTH for DIC score is not sensitive in the fibrinogen domain because the physiologic fibrinolysis terminated at the early stage but hyperfibrinolysis developed in the late stage of patients with COVID-19 infection.

3. Low anti-thrombin levels were reported and hyperfibrinogenemia might be apparent, both raising the concern that prophylactic dose of heparin or LMWH might be inadequate (123).

9. Despite VTE prophylaxis, the incidence of thrombosis remains high

In eight of ten cases autopsied in Brazil, pulmonary histology revealed variable numbers of small fibrinous thrombi in small pulmonary arterioles. A large number of pulmonary megakaryocytes were observed in the pulmonary capillaries, indicating activation of the coagulation cascade together with increased megakaryocytes in glomeruli and superficial dermal vessels.

The biopsy of purpuric skin lesions of three proven severe COVID-19 cases with ARDS showed pauciinflammatory thrombogenic vasculopathy (124). In three cases, minimally invasive autopsies were attempted in multiple organs including the lungs, heart, kidneys, spleen, bone marrow, liver and others (107). Monocyte and lymphocyte infiltration in the pulmonary vasculature were found with congestion along with hyaline thrombi of vessels. Necrosis of parenchymal cells and hyaline thrombus formation in small vessels were observed in multiple organs without evidence of infection(125). The thrombosis in multiple organs was possibly due to local inflammation only or might imply a hypercoagulable status.

10. Impact of blood count abnormalities

10.1. Neutrophil count

Subsequent reports showed a trend of higher neutrophil count in patients who required ICU admission (ANC 4.2 vs 2.6 × 10^9/L, p = 0.17) (25). Thus, patients requiring ICU care developed neutrophilia during the hospitalization, with a median peak Absolute Neutrophil Count (ANC) of 11.6 × 10^9/L, compared to 3.5 × 10^9/L in the non-ICU group (P value < 0.001).Further, in a retrospective review of 25 patients who died with COVID19 in Wuhan the neutrophil count trended up prior to death in 87.5% of patents with evaluable data (126).

10.2. Lymphocyte count

In the report from China Medical Treatment Expert Group for COVID-19 83.2% had lymphocytopenia at hospital admission (9). Importantly, the lymphopenia is a consistent marker of poor prognosis. Thus, when comparing 109 patients who died in Wuhan versus 116 patients who recovered, the patients who died presented with a decreased lymphocyte count (0.63 vs 1.0 × 109/L) and decreased lymphocyte percentage (127). In a retrospective cohort study of 191 patients with 54 deaths, lymphocyte count was lowest at day 7 of illness onset in survivors and then improved, whereas severe lymphopenia was observed until death in non-survivors (8). Wang et al. reported that non-survivors developed more severe lymphopenia over time (128). Flow cytometry on peripheral blood lymphocytes of COVID-19 patients requiring ICU care showed significantly lower CD45+, CD3+, CD4+, CD8+, CD16+, and CD16/56+ counts, without an inversion of the CD4/CD8 ratio (25).

Interestingly, while the number of CD4+ and CD8+ T cells were reduced, both the proportion and number of B cells were not affected or even increased in most patients. Further, the producton of IFN-γ by CD4+ T cells and not CD8+ T cells or NK cells tended to be lower in severe cases. Finally, circulating CD8 + T cells contained high concentrations of cytotoxic granules including perforin and granulysin. All these data suggest a dysregulated immune system with overactivation of cytotoxic CD8 + T cells (129).

Potential mechanisms of lymphocytopenia may include direct infection of the lymphocytes by the virus, though the proportion of ACE2- positive lymphocytes is quite small. Lymphocytes express the coronavirus receptor Angiotensin converting enzyme (ACE2)(130),(131).

10.3. Neutrophil to Lymphocyte Ratio (NLR)

An increased NLR at presentation has a strong association with increased disease severity when compared to patients without severe disease at presentation. Moreover, when stratified by high NLR (> 3.13) and age ≥ 50, 50% of the patients had severe illness. Similarly, in another analysis of 96 patients, Yang, et al. identified that 46.1% of non-severe patients with an NLR > 3.3 and age > 49.5 would transform into severe cases within a mean of 6.3 days (132). In a study of 301 patients, an NLR of 2.973 (AUC 0.7338, sensitivity 75.8%, specificity 66.8%) was associated with progression of disease (133). Finally, a meta-analysis of 5 studies from China with 828 patients, NLR was found to increase significantly in patients with severe disease (standardized mean difference = 2.404, 95% CI - 0.98-3.82) (26). Increased NLR was also associated with VTE with a mean NLR of 9.5 (5.9-13) in 33 patients who developed VTE versus 5 (3.5-7.9) in 165 patients without VTE (58).

10.4. Platelets

Thrombocytopenia was more pronounced in patients with severe infection with a mean platelet count of 137 × 109 /L vs 172 × 109 /L in non-severe patients. In a retrospective review specifically investigating the relationship between thrombocytopenia and mortality of 1476 consecutive patients in Jinyintan Hospital, Wuhan, thrombocytopenia was reported in 20.7% of patients, using a cutoff of 125 × 109 /l. 72.7% of non-survivors had < 125 × 109 /L platelets vs only 10.7% in survivors, $p < 0.001$(27). 76 patients (5.1%) had a platelet nadir < 50 × 109 /L with a mortality rate of 92.1%. The mortality rate was 61.2% in the group of patients with a nadir platelet count between 50 and 100 × 109 /L. Overall, the majority of patients appear to have a mild thrombocytopenia, which is more pronounced with severe infection. Presently, it is unclear if a decreased platelet count reflects a more severe DIC and increased consumption or a direct platelet-viral interaction(134). Multiple possible mechanisms are possible for viral infection induced thrombocytopenia. These include the development of autoantibodies and immune complexes mediating clearance; direct infection of hematopoietic stem/progenitor cells and the megakaryocytic lineage via CD13 or CD66a resulting in decreased production of platelets; and pathologic activation of the coagulation pathway and consumption of platelets (134),(135).

10.5. Hemoglobin

There has been a trend of worse anemia in patients with more severe disease,(25), with a median hemoglobin (Hgb) of 13.2 g/dL in patients requiring ICU versus 14.2 g/dL in non ICU patients ($p = 0.07$), and the majority of patients presented with a normal Hgb count. Of 1099 patients with confirmed COVID-19 in China, the median Hgb was 13.5 g/dL in non-severe patients and 12.8 g/dL in severe patients (9). However, this trend was not appreciated in a review of 393 patients who required or did not require invasive ventilation in New York City(136).

Table 1
Summary of literature on the impact of common hematologic parameters on disease severity in COVID-19

Lab	Location (reference)	N (total, non-severe/severe)	Non-severe	Severe	P value
White blood cell count ($\times 10^9$/L) Median (IQR) or [SD]	China (Guan, et.al NEJM Wuhan, China (Qin, et al. Clin Inf Disease) China (Wang et al. JAMA) Shangai, China (Wu, et al. Jama) Wuhan, China (Chen, et al. BMJ) Wuhan, China (Zhou, et.al)	1099, 926/174 452, 166/286 138, 102/36 201, 117/84 247, 161/113 191, 137/54	4.9 (3.8–6.0) 4.9 (3.7–6.1) 4.3 (3.3–5.4)a 5.02 (3.37 –7.18)b 5.0 (3.7–6.3)c 5.2 (4.3–7.7)c	3.7 (3.0–6.2) 5.6 (4.3–8.4) 6.6 (3.6–9.8)a 8.32 (5.07–11.20)b 10.2 (6.2–13.6)c 9.8 (6.9–13.9)c	NR < 0.001 0.003 < 0.001 NR < 0.0001
Absolute neutrophil count ($\times 10^9$ /L)Median (IQR) or [SD]	Wuhan, China (Qin, et al. Clin Inf Disease) China (Wang et al. JAMA) Shangai, China (Wu, et al. Jama) Wuhan, China (Chen, et al. BMJ)	452, 166/286 138, 102/36 201, 117/84 247, 161/113	5.2 (4.3–7.7)c 3.2 (2.1–4.4) 2.7 (1.9–3.9)a 3.06 (2.03–5.56)b	4.3 (2.9–7.0) 4.6 (2.6–7.9)a 7.04 (3.98–10.12)b 9.0 (5.4–12.7)c	< 0.001 < 0.001 < 0.001 NR
Absolute lymphocyte count ($\times 10^9$ /L) Median (IQR) or [SD]	China (Guan, et.al NEJM) Wuhan, China (Qin, et al. Clin Inf Disease) China (Wang et al. JAMA) Shangai, China (Wu, et al. Jama) Wuhan, China (Chen, et al. BMJ) Wuhan, China (Zhou, et.al)	1099, 926/174 452, 166/286 138, 102/36 201, 117/84 247, 161/113 191, 137/54	1.0 (0.8–1.4) 1.0 (0.7–1.3) 0.9 (0.6–1.2)a 1.08 (0.72–1.45)b 1.0 (0.7–1.4)c 1.1 (0.8–1.5)c	0.8 (0.6–1.0) 0.8 (0.6–1.1) 0.8 (0.5–0.9)a 0.67 (0.49–0.99) 0.6 (0.4–0.7)c 0.6 (0.5–0.8)c	NR < 0.001 0.03 < 0.001 NR < 0.0001
Neutrophil/lymphocyte ratio	Beijing, China (Liu, et al. preprint) Wuhan, China (Qin, et al. Clin Inf	61, 44/17 452, 166/286	2.2 (1.4–3.1) 3.2 (1.8–4.9)	3.6 (2.5–5.4) 5.5 (3.3–10.0)	0.003 < 0.001

Median (IQR) or [SD]	Disease) China (Yang, et al. Int Immun) Wuhan, China (Ma. et al.)	 93, 69/24 37, 17/20	 4.8 [± 3.5] 2.6 (1.8–3.5)e	 20.7 [± 24.1] 5.5 (3.6–6.5)e	 < 0.001 0.022
Platelet count (×109 /L) Median (IQR) or [SD]	China (Guan, et.al NEJM) China (Wang et al. JAMA) Shangai, China (Wu, et al. Jama) Wuhan, China (Chen, et al. BMJ) Wuhan, China (Zhou, et.al)	1099, 926/174 138, 102/36 201, 117/84 247, 161/113 191, 137/54	172 (139–212) 165 (125–188)a 178 (140.0–239.5)b 198 (160–256)	137 (99–179.5) 142 (119–202)a 187 (124.5–252.5)b 156 (111.8–219.3)c 165.5 (107–229)c	NR 0.78 0.73 NR < 0.0001
Hemoglobin (g/dL) Median (IQR) or [SD]	China (Guan, et.al NEJM) Wuhan, China (Chen, et al. BMJ) Wuhan, China (Zhou, et.al) New York, USA (Goyal et al. NEJM)	1099, 926/174 247, 161/113 191, 137/54 393, 263/130	13.5 (12.0–14.8) 12.8 (11.8–13.8)c 12.8 (12.0–14.0)c 13.5 (12.4–14.8)d	12.8 (11.2–14.1) 12.8 (11.4–14.5)c 12.6 (11.5–13.8)c 13.7 (12.3–15.3)d	NR NR 0.3 NR

NR = not reached.
a ICU vs non-ICU.
b Without ARDS vs with ARDS.
c Survivors vs Non-Survivors.
d Non-invasive vs invasive ventilation.
e Cancer patients.

Table 2
Summary of current literature evidence on the prognostic value of elevated D-dimer in COVID-19 across the globe.

Location (first author)	Sample size	Clinical setting	D-dimer assay (reference range)	D-dimer cut-off for risk assessment	Outcome of interest	Statistics (sensitivity/ specificity/odds ratio with pvalue)	Salient findings
Wuhan, China (Zhou et al)	191	Hospitalized	Unknown	> 1 µg/mL	Mortality	OR 18.42, 95% CI: 2.64-128.55; p = 0.0033	D-dimer > 1 µg/mL indicative of higher odds of death
Wuhan, China (Yao et al)	248	Hospitalized	Immunoturbidimetric assay (0-0.50 mg/L)	> 2.14 mg/L	Mortality	Se 88.2%/Sp 71.3%	D-dimer elevated in 74.6% of inpatients. Median D-dimer 6.21 mg/L and 1.02 mg/L in non-survivors and survivors respectively, p = 0.000
Wuhan, China (Zhang et al)	343	Hospitalized	CS5100 automatic coagulation analyzer (0-0.5 µg/mL)	> 2 µg/mL	Mortality	HR 51.5, p < 0.001; adjusted HR 22.4 (for age, gender and comorbidity), p = 0.003	D-dimer > 2.0 µg/mL had higher incidence of mortality when compared to < 2 (12/67 vs 1/267, P < 0.001)
Wuhan, China	183	Hospitalized	STA-R MAX coagulation analyzer	N/A (continuous variable)	Mortality	N/A	Median D-dimer values were 2.12 µg/mL vs 0.61

(Tang et al)							µg/mL in the non-survivors and survivors respectively, p < 0.001. 71.4% of non-curvivors had DIC per ISTH criteria
Mainland China (Guan et al)	1099	Hospitalized	Not mentioned	N/A (continuous variable)	Severe disease; Primary composite endpoint was admission to ICU/mechanical ventilation or death	N/A	1) 59.6% of the severe cases presented with elevated Ddimer vs 43.2% of non-severe cases (p = 0.002). 2) 69.4% of patients with the composite primary endpoint had elevated D-dimer vs. 44.2% of those without (P = 0.001)
Wuhan, China (Huang et al)	41	Hospitalized	Not mentioned	N/A (continuous variable)	ICU admission	N/A	Median D-dimer values were 2.4 vs 0.5 in the ICU patients and non-ICU patients respectively, p = 0.0042.
Wuhan, China (Wang et al)	138	Hospitalized	Not mentioned (0-500 mg/L)	N/A (continuous variable)	ICU admission	N/A	Median D-dimer values were 414 mg/L vs 166 mg/L, p < 0.001 in ICU cases and non-ICU cases respectively.
Wuhan, China (Wu et al)	201	Hospitalized	Not mentioned	N/A (continuous variable)	ARDS; mortality	ARDS HR = 1.03, p < 0.001; mortality HR = 1.02, p = 0.002	Higher D-dimer associated with progress to ARDS and mortality
Milan, Italy (Lodigiani et al)	388	Hospitalized	Not mentioned	N/A (continuous variable)	ICU; mortality	N/A	the higher Ddimer values in non-survivors vs survivors and also in ICU patients vs general ward patients.

Beijing, China (Cui et al)	81	ICU	Succeeder SF8200 automatic coagulation analyzer	> 1.5 µg/mL	VTE	Se 85%/Sp 88.5%/NPV 94.7%	20/81 (25%) patients had VTE. 8/20 patients with VTE died. D-dimer values were 5.2 ± 3.0 vs 0.8 ± 1.2 µg/ml in the VTE group and non-VTE group respectively, P < 0.001.
Strasbourg, France (Leonard-Lorant et al)	106	Hospitalized	Unknown	> 2660 µg/L	Pulmonary embolism	Se 100%/Sp 67%	32/106 (30%) patients had a PE. Median D-dimer values were IQR 6110 ± 4905 versus 1920 ± 3674 µg/L in the PE and non-PE group respectively, p < 0.001

11. Risk of VTE in COVID-19

Patients with COVID-19 have a relatively prolonged disease, with a duration that ranges between 17 and 25 days. (8). Although most patients have a favorable prognosis, older patients and those with chronic underlying conditions may have worse outcomes. The average death rate worldwide is about 6.6% (137). About one third of the worsened patients (8),(138). and up to 41.8% (139), suffer from complications such as respiratory failure, acute respiratory distress syndrome (ARDS). In addition, they may have heart failure, secondary bacterial infections, and septic shock.

During their illness the patients may require high-flow oxygen, inhalations, vasopressors, mechanical ventilations, and even ECMO. The complexity of disease in many patients necessitates a large number of highly qualified medical personnel to deal with these unique challenges.

12. Incidence of VTE in Patients with COVID-19

Patients with COVID-19 infection are at high risk of developing thromboembolic complications. Klok et al. (12), reported a high incidence of VTE in critically ill patients admitted to the ICU despite the use of at least prophylactic anticoagulation. They evaluated 184 ICU patients – mean age 64 ± 12 years, 139 (76%) of which were male, from 2 Dutch university hospitals and 1 Dutch teaching hospital, positive to COVID-19. Of these patients, 23 died (13%), 22 were discharged alive (12%), and 139 (76%) were still in the ICU on April 5, 2020. Computer tomography pulmonary angiogram (CTPA) and/or ultrasonography done by clinical suspicion confirmed VTE in 27% (95% CI 17–37%) and arterial thrombotic events in 3.7% (95% CI, 0–8.2%). PE was the most frequent thrombotic complication (n = 25, 81%). Age (adjusted hazard ratio 1.05/year, 95% CI 1.004 -1.01) and coagulopathy, defined as spontaneous prolongation of the prothrombin time >3 s or activated partial thromboplastin time >5 s (adjusted

hazard ratio 4.1, 95% CI 1.9–9.1), were independent predictors of thrombotic complications. None of the patients developed disseminated intravascular coagulation (140).

Cui et al. (22), described the course of 81 patients diagnosed with COVID-19 pneumonia in the ICU of Tongji Medical College, Huazhong University of Science and Technology. Their mean age was 59.9 years (range 32–91 years) and 37 (46%) were male; 64 (79%) patients have been discharged from the hospital, 8 (10%) had died, and the rest 9 (11%) remained hospitalized. No preventive anticoagulation was administered; 20/81 patients (25%) developed lower-extremity venous thrombosis.

However, patients with DVT were confirmed, and these were older (68.4 ± 9.1 vs. 57.1 ± 14.3 years, $p < 0.001$), had lower lymphocyte counts (0.8 ± 0.4 vs 1.3 ± 0.6 ×109 /L, $p < 0.001$), longer APTT (39.9 ± 6.4 vs. 35.6 ± 4.5 s, $p = 0.001$), and higher D-dimer (5.2 ± 3.0 vs. 0.8 ± 1.2 μg/mL, $p < 0.001$). Cui et al. (22), also showed that if a cut-off value of 1.5 μg/mL D-dimer was used to predict VTE, the sensitivity was 85.0%, the specificity was 88.5%, and the negative predictive value was 94.7%In contradictory fashion, Yao et al. (141), from Renmin Hospital of Wuhan University (Wuhan, China) reported that D-dimer elevation (≥0.50 mg/L) upon admission was present in 74.6% (185/248) of patients with COVID-19 in whom VTE was theoretically ruled out. However, ruling out VTE in their trial was based mainly on the Wells score, while Doppler ultrasound and CTPA was performed in only 4 patients with a high clinical suspicion for VTE. In the report, D-dimer was associated with both increased disease severity and in-hospital mortality. A D-dimer level of >2.14 mg/L predicted in-hospital mortality with a sensitivity of 88.2% and specificity of 71.3% (142),(143).

13. Thrombotic manifestations associated with COVID-19 infection

From clinical experience, several clotting events in central venous catheters have been noted. Some experience unusual thrombotic complications such as ischemic limbs, stroke and venous thrombo-embolism (VTE). Lower extremity deep vein thrombosis (DVT) has an incidence of approximately 25% without VTE prophylaxis(22). Of 183 cases reported in Tongji Hospital, Wuhan Province, 71.4% of nonsurvivors met the International Society on Thrombosis and Hemostasis (ISTH)'s diagnostic criteria for disseminated intravascular coagulation (DIC) resulting in high mortality rates (121). The median time of DIC status was 4 days.

Significantly higher D-dimer, fibrin degradation product (FDP), and prothrombin time (PT) were observed in the non-survivors group as compared with those in the survivors group. D-dimer was a good predictor of VTE. When D-dimer level is >1.5 μg/mL, patients have a risk of VTE showing a sensitivity of 85%, specificity of 88.5%, negative predictive value (NPV) of 94.7% and positive predictive value (PPV) of 70.8%. When low molecular weight heparin (LMWH) was initiated at a dose of 0.6 mg/kg every 12 hours, all patients demonstrated decreased D-dimer levels (144). Mean D-dimer can be used not only to diagnose thrombosis but is valuable in predicting the effectiveness of anticoagulants. The thrombotic complications among 184 patients in intensive care units of three Dutch hospitals; However, all patients received standard dose VTE prophylaxis using nadroparin. Still, thrombosis occurred as high as 31% (95%CI: 20- 41%) and comprised imaging-confirmed VTE in 27% (95%CI: 17-37%) and arterial thrombosis in 3.7% of cases (95%CI: 0-8.2%). Pulmonary embolism (PE) was the most frequent thrombotic complication (81%). These data indicated that a dose adjustment required.

Table 3 Clinical and laboratory parameters in survivors and nonsurvivors groups of COVID-19 infection

Parameters	Normal range	Total (n = 183)	Survivors (n = 162)	Non survivors (n = 21)	p values
Age (years)		54.1 ± 16.2	52.4 ± 15.6	64.0 ± 20.7	< 0.001

Underlying disease		75 (41.0%)	63 (38.9%)	12 (57.1%)	0.156
PT (sec)	11.5-14.5	13.7 (13.1-14.6)	13.6 (13.0-14.3)	15.5 (14.4-16.3)	< 0.001
APTT (sec)	29.0-42.0	41.6 (36.9-44.5)	41.2 (36.9-44.0)	44.8 (40.2-51.0)	0.096
Fibrinogen (g/L)	2.0-4.0	4.55 (3.66-5.17)	4.51 (3.65-5.09)	5.16 (3.74-5.69)	0.149
D-dimer (µg/mL)	< 0.50	0.66 (0.38-1.50)	0.61 (0.35-1.29)	2.12 (0.77-5.27)	< 0.001
FDP (µg/mL)	< 5.0	4.0 (4.0-4.9)	4.0 (4.0-4.3)	7.6 (4.0-23.4)	< 0.001
AT (%)	80-120	91 (83-97)	91 (84-97)	84 (78-90)	0.096
PTT, activated partial thromboplastin time; AT, antithrombin; FDP, fibrin degradation product; PT, prothrombin time					

14. Difficulties in the Diagnosis of VTE in Patients with COVID-19

Patients presenting with COVID-19 typically have a long course of disease, lasting up to several weeks (8). Some will need high oxygen supply and others will be intubated or treated with vasopressors. During this extended period of time, signs, symptoms and laboratory tests pointing to the diagnosis of VTE can be masked and attributed to COVID-19 or other complications occurring in the prolonged and sometimes complex hospitalization. D-dimer is increased in most of the patients, and usually cannot eliminate the presence of VTE. Therefore, timely diagnosis of VTE is anticipated to pose a significant challenge.

The clinical signs and symptoms of acute PE, the most menacing VTE event, are nonspecific (145),(146). Some of the patients are asymptomatic and others have dyspnea, chest pain, hemoptysis, presyncope or syncope, and up to hemodynamic instability. They can overlap with the symptoms of COVID-19 infection or its associated complications, such as ARDS, pleural effusion, or myocarditis (147),(8),(138),(139).

COVID-19 patients may have predisposing factor, such factors at baseline or acquire them during their illness and hospitalization – older age, elevated CRP, D-dimer, fibrinogen levels, tachypnea, fever, critical illness, infectious etiology, and immobility (145, 146, 148, 149). Risk scores such as the Wells score or the Geneva clinical prediction score useful, sparing unnecessary tests when predicting low risk for VTE. Hypoxemia, ECG changes indicating RV strain, sinus tachycardia, or atrial fibrillation present but they are not specific for PE and can be attributed to other complications of COVID-19

The diagnostic approach of PE (145),(146), is different between unstable and stable patients. The workup of hemodynamically unstable patients requires CTPA which is the method of choice for imaging. Mobilizing critically ill patients outside of the ICU for imaging burdensome and puts both the patients and their surroundings at additional risk of contamination. Transthoracic echo (TTE) and troponin levels are also part of the workup of unstable patients. TTE may indicate RV overload and/or dysfunction or even a right-sided thrombus. Nevertheless, elevated troponin is present in many of the patients positive to COVID-19 and indicates a severe infection (8),(16). In many cases it is a marker of myocardial injury among these patients (150),(151). Therefore, in the COVID-19 patient, an abnormal echocardiography

test or high troponin levels can also be attributed to the severe pulmonary disease, the “cytokine storm” associated with the viremia, and its systemic effects. It is therefore crucial to note that while both TTE and troponin levels can assist in reaching the diagnosis of PE, one needs a high level of suspicion in order to actively search for VTE and perform the confirmatory tests. Stable patients with suspected PE can be initially evaluated with a D-dimer test if they are at low or intermediate clinical probability for VTE. However, many of the COVID-19 patients may present with high levels of D-dimer due to other causes – inflammation, disseminated intravascular coagulation, advanced age, or infection(152),– suggesting the need for CTPA as an initial rule-out test as well. Nonetheless, as suggested by Yu et al.(153), who showed that D-dimer levels were significantly correlated with inflammation, in cases where the inflammatory state subsides, when the levels of D-dimer are non-proportionally high, VTE should be considered

V/Q scintigraphy (ventilation/perfusion lung scan) is also a feasible part of the workup in stable cooperative patients with suspected PE. However, this test exposes nuclear medicine workers to aerosolized secretions due to leakage of the aerosol to the room and because the patients frequently cough after inhalation. Using a N-95 mask may reduce the risk of infection, and it is possible to perform perfusion scans only (without the ventilation part), but choosing CTPA as the foremost imaging modality for a better option (154).

Finally, compression ultrasonography shows DVT only in 30–50% of patients with PE, but has a sensitivity of >90% and a specificity of ≥95% for proximal symptomatic DVT (155), in which case it is a class I indication for the diagnosis of PE (145). This test must therefore be considered in patients with COVID-19 as well, in appropriate cases.

As for the diagnosis of DVT in COVID-19 patients, a low D-dimer level may help in ruling out the diagnosis. Nevertheless, most patients cannot be regarded as low probability risk, and if clinically suspected, compression ultrasonography is the method of choice, starting from proximal ultrasound and if negative performing wholeleg ultrasound with a sensitivity of 96–99% and specificity of 99.8% for lower-extremity DVT (156).

COVID-19 is very contagious with an average reproduction number (R0) of 3.28 (157), which indicates the transmissibility of the virus by an infectious person in a totally naive population. This complicates the workup of patients, when compared with other patients who are not infected. Every single physical examination, laboratory test, or imaging examination of the patients, including CTPA or echocardiography, requires the full protection of the staff. In addition, all machinery must go through sterilization after use – a process which is time-consuming and may add hardship to what is an already strained health care system. This makes every contact with the patients bothersome, as well as precarious, for both the medical team and other patients within the medical facility.

15. Full blood count and biochemistry findings: correlation with prognosis

Following viremia, SARS-CoV-2 primarily affects the tissues expressing high levels of ACE2 including the lungs, heart and gastrointestinal tract. Approximately 7 to 14 days from the onset of the initial symptoms, there is a surge in the clinical manifestations of the disease. This is with a pronounced systemic increase of inflammatory mediators and cytokines, which characterized as a “cytokine storm.”(158). At this point, significant lymphopenia becomes evident. It has been shown that

lymphocytes express the ACE2 receptor on their surface (131), thus SARS-CoV-2 may directly infect those cells and ultimately lead to their lysis. Furthermore, the cytokine storm is characterized by markedly increased levels of interleukins (mostly IL-6, IL-2, IL-7, granulocyte colony stimulating factor, interferon-γ inducible protein(159), MCP-1, MIP1-a) and tumor necrosis factor (TNF)-alpha, which may promote lymphocyte apoptosis (159),(160),(161), Substantial cytokine activation also associated with atrophy of lymphoid organs, including the spleen, and further impairs lymphocyte turnover (162). Coexisting lactic acid acidosis, which more prominent among cancer patients who are at increased risk for complications from COVID-19, (163), may also inhibit lymphocyte proliferation (164).

Guan et al. provided data on the clinical characteristics of 1099 COVID-19 cases with laboratory confirmation during the first 2 months of the epidemic in China (9). On admission, the vast majority of patients presented with lymphocytopenia (83.2%), whereas 36.2% had thrombocytopenia, and 33.7% showed leukopenia. These hematological abnormalities were more prominent among severe vs non-severe cases (96.1% vs 80.4% for lymphocytopenia, 57.7% vs 31.6% for thrombocytopenia, and 61.1% vs 28.1% for leukopenia). These results were consistent in four other descriptive studies that were conducted during the same period in China and included 41, 99, 138 and 201 confirmed cases with COVID-19, respectively (10),(138),(165),(166). Specifically, Huang et al.,17 and Wang et al.19 highlighted an association between lymphopenia and need of ICU care, whereas Wu et al.(166), showed an association between lymphopenia and acute respiratory distress syndrome (ARDS) development. Specifically, Wu et al. retrospectively analyzed possible risk factors for developing ARDS and death among 201 patients with COVID-19 pneumonia in Wuhan, China (166). Increased risk of ARDS during the disease course was significantly associated with increased neutrophils ($P < .001$), decreased lymphocytes ($P < .001$) in a bivariate Cox regression analysis. Increased neutrophils ($P = .03$) were associated with increased risk of death (166).

Furthermore, lymphopenia and was also documented in approximately 40% of the first 18 hospitalized patients with COVID-19 in Singapore (167). A more recent report on 69 patients confirmed the percentage of those with lymphocytopenia, whereas 20% had mild thrombocytopenia (25). Interestingly, 69% of patients with a low lymphocyte count showed a reactive lymphocyte population including a lymphoplasmacytoid subset, which was not common in the peripheral blood of patients with SARS infection in 2003 (25, 168, 169). Flow cytometry did not reveal any inversion in the CD4+/CD8+ lymphocyte ratio (25). However, functional studies have suggested that SARS-CoV-2 may impair the function of CD4+ helper and regulatory T-cells and promote the initial hyperactivation which is followed by rapid exhaustion of cytotoxic CD8+ T-cells (170),(171).

In Singapore, Fan et al. also found that patients requiring ICU support had significantly lower lymphocyte levels ($P < .001$) at baseline (25). In another retrospective study including 52 critically ill patients from Wuhan, China, lymphopenia was reported in 85% of patients.27m Lymphopenia was also prominent among critically ill patients with COVID-19 in Washington, USA (172),(173). During hospitalization, nonsurvivors demonstrated a more significant deterioration in lymphopenia compared with those who survived ($P < .05$) (165). It has also been reported that patients with severe disease and fatal outcomes present with a decreased lymphocyte/white blood cell ratio both in admission ($P < .001$) and during hospitalization ($P < .001$) compared with those who survived. (171),(127). Contrary to non-survivors, survivors demonstrated a nadir of lymphocytes counted on day 7 from symptom onset and a subsequent

restoration (8). Therefore, serial assessment of lymphocyte count dynamics may predictive of patient outcome (130).

Recent studies have shown that myocardial injury among inpatients with COVID-19 is associated with increased mortality (151),(174). In a prospective study in Wuhan, China including 416 consecutive patients 82 (19.7%) had documented myocardial injury. Compared with the others, these patients with myocardial injury had higher leukocyte ($P < .001$), lower lymphocyte ($P < .001$) and lower platelet counts ($P < .001$) (151). A retrospective study including 187 patients with COVID19 from another hospital in Wuhan showed that patients with high troponin-T levels had leukocytosis ($P < .001$), increased neutrophils ($P < .001$) and decreased lymphocytes ($P = .01$) (174).

A meta-analysis of nine studies has suggested that thrombocytopenia is significantly associated with the severity of the COVID-19 disease, with very high between-studies heterogeneity though; a more sizeable drop in platelet counts was noted especially in non-survivors (175). Qu et al showed that among 30 hospitalized patients with COVID-19, those presenting with a peak in the platelet count during the disease course had worse outcomes (176). Interestingly, the platelet to lymphocyte ratio at the time of platelet peak emerged as an independent prognostic factor for prolonged hospitalization in the multivariate analysis. It was suggested that a high platelet to lymphocyte ratio may indicate a more pronounced cytokine storm, due to enhanced platelet activation (176).

16. HEMATOLOGIC PAR AMETERS OF PATIENTS WITH COVID-19 INFECTION

Although information is in some cases based on the results of limited amount of data and should be validated with additional studies, the available findings clearly establish the clinical hematology laboratory as an important partner in the triage and management of affected patients. Apart from RT-PCR testing for the organism, laboratory tests have not been assessed with regard to their sensitivity or specificity for the diagnosis of COVID-19, although their value as prognostic indicators has been established. A summary of the major hematologic features of importance in COVID-19-infected patients follows.

16.1. Lymphopenia

Lymphopenia is a common finding in patients with COVID-19 infection and is believed to represent a defective immune response to the virus (11). In their early study of 41 adults with RT-PCR–confirmed COVID-19 infection, Huang et al noted that lymphopenia (defined as an absolute lymphocyte count <1.0 × 109 /L) was seen in 26 (63%) of patients.(161). A recent meta-analysis noted that 35%-75% of patients developed lymphopenia, which was a more frequent feature of patients who died of disease (162). In their analysis of 67 COVID-19 patients from Singapore, Fan et al (163), identified an lymphocyte count of $<0.6 \times 10^9$/L being predictive for admission to the intensive care unit (ICU). The apparent viral genomic mutations, it is possible that the immunologic response to the virus may change as the pandemic expands into other countries. Another possibility is that testing of patients is nonuniform and, depending on the time of presentation, the extent of lymphopenia may vary. A careful review of reported data for these issues is therefore recommended. In children, lymphopenia is much less common. In their meta-analysis of 66 cases reported in the Chinese literature, Henry et al (10), identified lymphopenia in 3% of patients. This is in contrast to other similar viral infections, such as SARS, in which lymphopenia was a much more common finding in children.

16.2. Leukocytosis

A meta-analysis of the extant literature noted that leukocytosis was identified in 11.4% of patients with severe disease compared to 4.8% of patients with mild-to-moderate disease (odds ratio [OR], 2.54; 95% confidence interval [CI], 1.43-4.52) (11).

16.3. Neutrophilia

The data on neutrophilia are incomplete and have not been widely addressed in the literature. The available data suggest that neutrophilia is an expression of the cytokine storm and hyperinflammatory state which have an important pathogenetic role in COVID-19 and related infections such as SARS (161),(138, 165, 166). Cytoplasmic and nuclear morphological anomalies, from hyposegmented nuclei to apoptosis, have been described in circulating granulocytes at the time of hospital admission, possibly in relation with the hyperinflammatory state with cytokine storm. They usually precede the increase of reactive lymphocytes (167). Neutrophilia may also indicate superimposed bacterial infection (11). Fan et al noted that neutrophilia is common in patients treated in the ICU during hospitalization (11.6 vs 3.5 × 109 /L) (163).

16.4. Markers of systemic inflammation

A number of biomarkers of systemic inflammation including sepsis have become available as reportable elements of the major commercially available blood analyzers as part of the expanded CBC or as parameters measured in research mode. Among these are neutrophil CD64 expression, mean cell volume of neutrophils and monocytes, immature granulocyte fraction, delta neutrophil index, and monocyte distribution width (MDW) (11). The potential application of data derived from the CBC would be to use formulas such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio, and monocyte-to-lymphocyte ratio to act as surrogates to assess the extent of systemic inflammation. Although extensive study is at this point lacking, Qin et al20 have reported an increase in NLR in patients with severe disease compared to those without.

16.5. Thrombocytopenia

A meta-analysis pooling data from nine studies showed that thrombocytopenia has been reported in a majority of patients. This is similar to data reported in the SARS outbreak, in which thrombocytopenia was reported in ~55% of cases and correlated with increased risk of severe disease (25, 168, 169). In patients with severe infection, thrombocytopenia is identified in up to 57.7% of patients vs 31.6% of patients with less significant COVID-19 symptoms (OR 2.96, 95% CI, 2.07-4.22) (11). Interestingly, thrombocytosis has been identified in a minority of patients, for example by Chen et al who report this finding in ~4% of cases (138).

The use of platelet count in conjunction with other factors associated with severe disease has to our knowledge not been reported for COVID-19 patients, although it has been revealed to be of use in SARS. For example, Zou et al reported that platelet count, in conjunction with hypoxemia, was used in prognostic model for SARS that predicted severe disease with 96.2% accuracy (168),(170).

16.6. Coagulation parameters

A subset of severe pneumonia patients develop viral sepsis, disseminated intravascular coagulation (DIC), and multiorgan failure (171). Coagulation parameters show abnormal results related to sepsis or DIC. Prothrombin time (PT), an assay used to evaluate the extrinsic and common coagulation pathways, and D-dimer are useful indicators of prognosis and severity of disease in COVID-19. (19).

In a study with 183 coronavirus pneumonia, PT, activated partial thromboplastin time (APTT), fibrinogen, antithrombin, fibrin degradation product (FDP) and D-dimer were consecutively measured during 2-week hospitalization. The overall mortality was 11.5%. The nonsurvivors demonstrated significantly higher D-dimer and FDP levels, and longer PT and APTT compared to survivors on admission. The fibrinogen and antithrombin levels were significantly reduced in nonsurvivors during hospitalization, and D-dimer and FDP are markedly elevated in all nonsurvivors by the late hospitalization, which suggested a common coagulation activation, dysregulated thrombin generation, impaired natural anticoagulants, and fibrinolysis (172),(173). In addition, several critically ill patients have been reported to develop coagulopathy, antiphospholipid antibodies, and increased arterial and venous thrombotic events such as cerebral infarction (127). Early recognition of these abnormal coagulation results will be useful to predict the disease severity, support to guide the therapy, and improve the patients' clinical outcome. (171).

TABLE 4 Hematologic biomarkers of importance in COVID-19 infection (adapted by the authors from Ref. 6). For details, see text

Parameter	Clinical significance	References
Lymphopenia	Defective host response	((177),(10),(178)
Leukocytosis	Bacteria superinfection	(177)
Neutrophilia	Bacterial superinfection, cytokine storm	(177),(10),(25),(138),(18),(179)
Thrombocytopenia	Consumptive coagulopathy	(177),(175),(180)
Abbreviation: MDW, monocyte volume distribution width.		

TABLE 5 Other laboratory biomarkers of importance in COVID-19 infection (adapted by the authors from Ref. 6). For details, see text

Parameter	Clinical significance	References
Increased CRP	Severe viral infection, including viremia	(177),(178),(181)
Increased procalcitonin	Bacterial superinfection	(177),(10),(178, 181)
Increased LDH	Pulmonary injury/multiorgan damage	(177),(178),(25)
Increased aminotransferases	Liver injury/multiorgan damage	(177),(178),(25)
Increased bilirubin	Liver injury	(177),(178)
Increased creatinine	Renal injury	(177),(178)
Increased cardiac troponins	Cardiac injury	(177),(182)
Decreased albumin Impaired	liver function	(177, 178)
Prolonged prothrombin time	Consumptive coagulopathy	(177),(183)
Prolonged APTT	Consumptive coagulopathy	(177),(183)
Increased D-dimer and/or FDP	Consumptive coagulopathy	(177),(183)
Abbreviations: APTT, activated partial thromboplastin time; CRP, C-reactive protein; FDP, fibrin		

degradation product; LDH, lactate dehydrogenase

17. VTE Prophylaxis in non-ICU Hospitalized COVID-19 Patients

Hospitalized acutely-ill medical patients, including those with infections such as viral pneumonia, are at increased risk for VTE, and antithrombotic practice guidelines recommend thromboprophylaxis with twice- or thrice-daily subcutaneous unfractionated heparin (UFH) oncedaily subcutaneous low-molecular-weight heparin (LMWH), or fondaparinux to reduce this risk, although fondaparinux is infrequently used due to its long half-life and reversibility concerns (184),(185).

Preliminary reports in patients with severe pneumonia due to COVID-19 as well as previous reports of severe pneumonias/severe acute respiratory syndromes from other viruses such as influenza H1N1 or Middle East respiratory syndrome (MERS-CoV) suggest a multi-fold higher risk for VTE and, in particular, in increased risk for PE (186). In addition, patient specific VTE risk factors such as advanced age, a prior history of VTE, a history of or active cancer, immobility, and thrombophilia, had been incorporated prior to the COVID-19 era to assess overall VTE risk using standardized VTE risk assessment scores such as Padua VTE or IMPROVE VTE risk scores(187),(188),(189), which had been externally validated(190-192). The optimal VTE risk stratification scheme for hospitalized COVID-19 patients requires further study, including the use of very elevated D-dimer levels (>6 times the upper limit of normal [ULN]) that appear to be a consistent predictor of thrombotic events and poor overall prognosis in this population (193).

All hospitalized patients with COVID-19 should be considered for thromboprophylaxis with either UFH or LMWH unless there are absolute contraindications. Advantages of LMWH over UFH include once daily versus twice or thrice daily injections and less heparin-induced thrombocytopenia. Although some DOACs are approved for in-hospital prophylaxis, these agents should be considered with caution in COVID-19 patients in whom co-administration of immunosuppressant, antiviral and other experimental therapies may potentiate or interfere with DOAC therapy (51). For patients in whom anticoagulant therapy is contraindicated, mechanical thromboprophylaxis, preferably with intermittent pneumatic compression devices, should be utilized, although there is limited evidence of efficacy in hospitalized medically ill patients (184),(194).

18. VTE Prophylaxis in ICU Hospitalized COVID-19 Patients

Hospitalized COVID-19 patients who are managed in an ICU or CCU setting have an overall poor prognosis, with the proportion of severe cases approaching 26% (95% CI: 17.4 -34.9) and reported case-fatality rates of 42% (9). The presence of co-morbid conditions (e.g., cardiovascular disease, obesity), a SIC score ≥4, and elevated levels of D-dimer (>6 times ULN), C-reactive protein and troponins, and other markers of disseminated intravascular coagulopathy (DIC) as assessed by the ISTH scoring system are associated with a worse prognosis (51),(150),(195). In addition, the heightened prothrombotic tendency in the critically ill hospitalized patients with COVID-19 pneumonia, leading to VTE and especially, in situ pulmonary artery microthrombi, is evident in case series and pathologic studies as an endpoint of pulmonary inflammation (196),(197). studies found an incidence of VTE and arterial thromboembolism of 27% and 3.7%, respectively, in 184 COVID-19 patients who were in an ICU setting and were receiving standard-dose thrombo-prophylaxis (198),(22). Lastly, the use of tissue plasminogen activator in the

treatment of COVID-19-associated ARDS was associated with only transient improvement of pulmonary function (199).

Emerging clinical data suggests that the use of either prophylactic to intermediate doses of LMWH (e.g., enoxaparin, 40-60 mg daily) in very sick COVID-19 patients (Ddimer >6 times ULN; SIC score ≥4) is associated with improved outcomes and a better prognosis (51). Expert clinical guidance statements and clinical pathways from large academic healthcare systems favor the use of standard-dose regimens with LMWH or UFH (especially for patients with a creatinine clearance < 30 mL/min), mechanical thromboprophylaxis (intermittent pneumatic compression) when anticoagulants were contraindicated, use of multimodal (anticoagulant and mechanical) prophylaxis strategies in the critically ill and completely immobile COVID-19 population (16, 122), and the use of VTE risk stratification using either clinical criteria (body mass index [BMI] >30 kg/m2), VTE risk scores and/or biomarkers (e.g., very elevated D-dimer levels) to suggest intermediate- or higher-dose LMWH or UFH regimens (e.g. enoxaparin 0.5 mg/kg twice-daily; enoxaparin 40 mg twice-daily, intravenous UFH targeted to an anti-factor Xa level of 0.30-0.70 IU/mL) (200). The use of empiric therapeutic-dose anticoagulation has been advocated by some for the critically ill hospitalized COVID-19 patients, especially in ICU settings;(198). However, there are ongoing randomized trials that aim to assess the efficacy and safety of more intense intermediate- to therapeutic-dose versus prophylactic-dose LMWH in hospitalized COVID-19 patients including COVID Hep (ClinicalTrials.gov Identifier: NCT04345848), Hep-COVID, and PROTECT COVID 19.

19. Hematologic parameters to predict COVID-19 prognosis

Prognosis may depend on multiple factors such as age and underlying disease but hematologic parameters also predict the mortality as well. Multivariate analysis of 28-day mortality in severe COVID-19 showed D-dimer, PT and platelet count correlated with mortality rate (51). Another study showed values to predict mortality included D-dimer level > 1 μg/mL, platelet count < 100 × 109/L, WBC count > 10 ×109/L, lymphocyte count < 0.8×109/L and PT ≥ 16 sec (31)

20. Anti-coagulation therapy

As COVID-19 infection is interestingly associated with arterial and venous thrombosis, all hospitalised patients without evidence of active bleeding should be given prophylactic anticoagulation (51). Mechanical thrombo-prophylaxis such as intermittent pneumatic devices (IPD) should only be used if pharmacological anticoagulation is contraindicated. The use of both concomitant pharmacological and mechanical anticoagulation should be avoided (51). Prolonged PT and aPTTm without evidence of bleeding should not preclude the use of anticoagulation. Patients who are on extracorporeal membrane oxygenation (ECMO) and/or continuous renal replacement therapy (CRRT) are believed have a higher risk of thromboembolism due to the increased inflammatory process(201). The anticoagulation of choice is low molecular weight heparin (LMWH), unfractionated heparin (UFH) or subcutaneous fondaprinux (202). Unfractionated heparin is a naturally occurring glycosaminoglycan with anti-thrombin and anti-inflammatory activity which has little interaction with drugs used to treat COVID-19 infection (8). Its short half-life favours the use in patients with high bleeding risk or renal impairment. Low molecular weight heparin such as enoxaparin 0.5 mg/kg once daily dosing is a better choice as they do not require frequent blood sampling (203). However, in patients with severe renal impairment (creatinine clearance <

30 ml/min/1.73 m2), LMWH requires a well-callibrated anti-Factor Xa assay monitoring to ensure efficacy and to avoid drug toxicity (204),(205).

Patients with atrial fibrillation, prosthetic cardiac valves and preexisting venous thrombosis who are currently treated with vitamin-K antagonist (warfarin) or direct oral anticoagulant (DOAC), it is important to note that these drugs may interfere with antiviral therapy used in COVID-19. In such a setting, an individual patient-based approach would be appopriate and a decision to be made to change the patient's existing treatment to a more convenient parenteral LMWH during the critical illness period (206).

The American Society of Hematology COVID-19 task force recommends that prophylactic anticoagulation should only be withheld in the presence of active bleeding or at a platelet count of less than 25 x 10^9/L. Meanwhile patients with AF, mechanical cardiac valves and preexisting thrombotic events should continue their full dose anticoagulation and are only advised to withhold such treatment at a platelet count of less than 30 x 10^9/L. (199).

Patients who are at high risk of developing thrombosis (Padua prediction score for risk of developing venous thromboembolism in hospitalised patients) such as; over 70 years of age, poor mobility, intensive care unit (ICU) admission, body mass index (BMI > 30 kg/m2) and history of active cancer should be given extended prophylactic anticoagulation upon discharge for a duration of 35–42 days (202). Direct oral anticoagulant (DOAC) such betrixaban 60 mg daily or rivaroxaban 10 mg daily would be the anticoagulants of choice. On the other hand, those who are low risk may receive only low dose aspirin prophylaxis (aspirin 81 mg twice daily) for not less than 4 weeks in duration from discharge (202).

21. Management of coagulopathy

Coagulopathy without active bleeding should not warrant any transfusion of blood products as injudicious transfusion may lead to respiratory compromise and adverse events(207). A patient with COVID-19 disease with bleeding episodes secondary to DIC should be treated like any other sepsis-induced DIC. They should receive red blood cells, platelet concentrates, cryoprecipitate (1 unit for every 10 kg per body weight) and virally inactivated plasma to maintain a platelet count of more than 50 x 10^9/L and a plasma fibrinogen level of 1.5 g/L (207). Antifibrinolytics such as tranexamic acid should be avoided in such situations because the excess fibrin need to be broken down. 4- factor prothrombin complex concentrate (4F-PCC) and fibrinogen concentrates would be the products of choice in coagulopathy associated with liver failure(208).

22. Management of thrombotic risk and bleeding episodes

The routine testing in a hematologic laboratory is indicated for all hospitalized COVID-19 cases The tests include complete blood count (CBC), coagulation studies, fibrinogen, and Ddimer for thrombotic evaluation reasons. Further, we recommend Repeated test is followed on a daily basis or less frequently as appropriate(209). A specific diagnosis test for DVT and PE is considered when signs/symptoms are present.

In all, 449 patients in Tongji Hospital had used anticoagulant treatment, mainly LMWH for 7 days or longer. No difference was observed in the 28-day mortality rate for user and nonuser groups (30.3 vs. 29%, p = 0.910). However, for subgroup analysis, when the severity is high enough (SIC score ≥ 4) or

high D-dimer level (more than 6 fold of the upper normal limit), use of anticoagulants showed more significant improvement in the 28-day mortality rate in the user than in the nonuser group [(high severity) 40.4% vs. 64.2, p = 0.029 and (high D-dimer) 32.8 vs. 52.4%, p = 0.017].

Regarding D-dimer ≤ 1 upper limit of normal, the 28-day mortality rate was higher in the treatment group compared with that of the nontreatment group but without statistical significance (33.3 vs 9.7%, p = 0.260). Therefore, anticoagulants have an advantage only in some exclusively selected cases(123).

Table 6 Comparison of different guidelines in anticoagulant treatment among patients with COVID-19

	ASH	ISTH	ACC
Evaluated risk of thrombo-embolism		All hospitalized patients	All hospitalized patients
Hospitalized			
- Prophylaxis dose	All cases	All immobilized and severely ill patients	All immobilized patients with respiratory failure, with comorbidities and those requiring intensive care
- Therapeutic dose without evidence of thrombosis	No adequate data		No adequate data
- Type of medication	LMWH, fondaparinux	LMWH, fondaparinux	LMWH
Mechanical prophylaxis	Only with pharmacological contra-indication	All cases completely immobilized combined with pharmacologic method	Only when there is pharmacologic contraindication
Postdischarge thromboprophylaxis	May consider DOACs		May consider (DOACs, LMWH
Management of hemorrhage		Replacement therapy along with tranexamic acid	Same as ISTH

ACC, American College of Cardiology; ASH, American Society of Hematology; ISTH, International Society on Thrombosis and Hemostasis; LMWH, low molecular weight heparin; DOAC, direct oral anticoagulants

When recurrent clotting occurs despite prophylactic therapy, increasing the intensity should be considered. Any acute medical illness carries an increasing risk for VTE for up to 90 days after discharge. Thus, despite inadequate data on postdischarge thromboprophylaxis, it can be prescribed using adjusted doses among individuals who are elderly, with comorbidities and with elevated D-dimer levels > 2 times the upper normal limit (209).

Pharmacological thromboprophylaxis should be given to all immobilized and severely ill patients, preferably LMWH or fondaparinux. All completely immobilized patients would benefit from pneumatic compression in addition to pharmacological thromboprophylaxis, differing from ASH recommendations.

Mechanical thromboprophylaxis should be used alone when platelet level is less than 30 $\times 10^9$/L. When sudden onset of desaturation or respiratory distress occurs, pulmonary VTE should be considered. When major bleeding is encountered, fresh frozen plasma, fibrinogen or platelet replacement therapy is indicated. Use of tranexamic acid is reasonable in major hemorrhages without increased thrombotic events if no DIC is present. Recombinant factor VII and prothrombin complex concentrate are not recommended (122).

According to the American College of Cardiology (ACC) recommendations, VTE risk stratification should be evaluated in all hospitalized patients. Hospitalized patients with respiratory failure and co-morbidities, immobilized or requiring intensive care, should receive pharmacological prophylaxis. According to WHO recommendation, LMWH and direct oral anticoagulants (DOACs) should be considered for postdischarge thromboprophylaxis because they can reduce the risk of VTE. However, they may increase bleeding events. Extended prophylaxis for up to 45 days is indicated only among patients with high VTE risk (especially those with comorbidities and elevated D-dimer > 2 times the upper normal limit) (210).

The complex relationship between thrombosis and inflammation may have some correlation and depend on multiple mediators working at different levels of the vascular system. Endothelium injury may also release P selectin and VCAM-1 to induce migration of leukocytes, initiating inflammation. Thrombin may directly stimulate an inflammatory response on endothelial cells and platelets and further lead to increasing pro-inflammatory cytokines (211). This effect can explain the beneficial effectiveness of anticoagulant therapy in severe COVID-19 cases (212).

Salvage therapy with fibrinolytic agents is now proposed as an expert opinion especially in massive pulmonarym VTE cases. Other indication includes limb-threatening DVT, acute stroke or acute myocardial infarction (120) Urgent thrombolysis with recombinant tissue plasminogen activator may indicated as a last chance for survival (123), along with therapeutic LMWH but unfractionated heparin (UFH) is more preferred in this setting due to concerns in bleeding complications.

23. Treatment of VTE Patients with COVID-19

Treatment of patients suffering from PE (145),(146),(213), in a similar fashion to the diagnosis of PE, is also different in unstable and stable patients. In the unstable patient, reperfusion therapy by thrombolysis and unfractionated heparin (UFH) is the treatment of choice. Nevertheless, many of the patients with COVID-19 have an absolute or a relative contraindication to thrombolysis, such as coagulopathy, thrombocytopenia, a recent invasive procedure, pericarditis, and age >75 years (8),(138),(214). Moreover, even those who are able to receive thrombolysis may require further invasive diagnostics or interventions regarding their COVID-19 infection which thrombolysis may prevent (such as insertion of central-line, pericardiocentesis, insertion of a chest tube, or performing ECMO).

One of the indications for PE as a cause for hemodynamic instability is the function of the RV. RV failure related to PE may also respond to a fluid challenge or to vasopressors, both of which may already be administered for some of the COVID-19 patients (157),(215).

The anticoagulation therapy of stable PE patients is usually LMWH or direct oral anticoagulants (DOACs). In patients with intermediate-/high-risk PE, UFH preferred due to its short half-life and the

opportunity to give the patients protamine sulfate as an antidote in case of the need for an urgent procedure or bleeding. Therefore, in COVID-19 patients, LMWH preferred. For practical reasons, some institutions will prefer DOACs in order to reduce the contact between the nurses and COVID-19 patients (146). However, drug interaction between DOACs and medical treatment for COVID-19 infection should be considered, as well as the potential risk of organ deterioration in these patients and lack of an effective reversal agent in some centers (216),(210). Inferior vena cava filters should be considered in selected patients with acute PE and absolute contraindications to anticoagulation, or in case of recurrence despite proper anticoagulant treatment (145).

The recent European Society of Cardiology (ESC) PE guidelines (145), emphasize the importance of induction of anticoagulation therapy in all patients suspected for PE with high or intermediate clinical probability of PE without delay, while diagnostic workup is in progress. These guidelines are most relevant in COVID-19 patients due to the very possible delay in diagnosis on account of protection of the medical team, sterilization of the machinery, and the difficulty in transporting hemodynamically unstable or intubated patients out of the ICU. As previously mentioned, patients with COVID-19 may present variably, from asymptomatic carriers up to a severe disease requiring admission to the ICU (8),(138),(138). Therefore, the treatment of VTE in these patients must be tailored personally, according to their infectious condition, VTE severity, and general status (146).

Table 7 Current guidelines and recommendations on prophylactic and therapeutic anticoagulation from different societies and institutions.

Recommending source	When to consider prophylactic dose anticoagulation	When to consider therapeutic dose anticoagulation
International Society of Thrombosis & Hemostasis	In all patients with COVID-19 who are hospitalized, including noncritically ill, in the absence of contraindications (active bleeding and platelet count < 25×10^9 /L). PT and PTT abnormalities are not considered a contraindication (201).	
American Society of Hematology (Expert Panel)	All hospitalized patients with COVID-19. LMWH or fondaparinux (suggested over UFH to reduce contact) in the absence of increased bleeding risk(217).	• Intubated patients who develop sudden clinical and laboratory findings consistent with PE, especially when chest X-ray and/or markers of inflammation are stable or improving • Patients with physical findings consistent with thrombosis, such as superficial thrombophlebitis, peripheral ischemia or cyanosis, thrombosis of dialysis filters, tubing or catheters, or retiform purpura • Patients with respiratory failure, particularly when D-dimer and/ or fibrinogen levels are very high, in whom other

		causes are not identified (e.g., ARDS, fluid overload)(218)
Thrombosis UK	• For CrCl > 30 mL/min: Give LMWH or fondaparinux • For CrCl < 30 mL/min or acute kidney injury: UFH 5000 units SC BD or TDS or dose-reduced LMWH • All completely immobilized patients would benefit from intermittent pneumatic compression in addition to pharmacological thromboprophylaxis • Mechanical thromboprophylaxis should be used alone if platelets < 30 × 10^9 /L or bleeding (122).	
National Institute for Public Health of the Netherlands	All patients with (suspected) COVID-19 admitted to the hospital, irrespective of risk scores.	• In patients with a D-dimer < 1,000 µg/L on admission but a significant increase during hospital stay to levels above 2,000- 4,000 µg/L, when imaging is not feasible, therapeutic-dose LMWH can be considered when the risk of bleeding is acceptable. • In patients with a strongly increased D-dimer on admission (e.g. 2,000-4,000 µg/L), D-dimer testing should be repeated within 24- 48 h to detect further increases in which case imaging for DVT or PE, or empiric anticoagulation, should be considered (219).

24. Duration of Thromboprophylaxis in Hospitalized COVID-19 Patients

At least 60% of all VTE events in medically ill patients occur in the post-hospital discharge period, with the first 3 weeks being associated with a greater than 5-fold increased risk in fatal PE (189). Earlier studies of extended thromboprophylaxis with DOACs revealed either limited efficacy or an increase in major bleed risk, and particularly due to these safety concerns, the most recent antithrombotic guidelines recommended against routine post-discharge thromboprophylaxis in medically ill patients, including those with pneumonia (185). However, more recent data reveals that in selected populations at high VTE risk and low bleed risk, based on key risk factors or risk models for thrombosis and bleeding, extended-duration thromboprophylaxis for approximately 4 weeks with prophylactic-dose LMWH (e.g., enoxaparin, dalteparin, tinzaparin) or a DOAC (e.g. rivaroxaban, betrixaban) provides a net clinic benefit by reducing VTE risk without incurring a significant increase in the risk of major bleeding (220),(221),(222). Recent data also supports that a modified IMPROVE VTE score using established cut-

offs plus elevated D-dimer (>2 times ULN) identifies patients at an almost three-fold higher risk for VTE in whom there is a significant benefit for extended-duration thromboprophylaxis (223). In the absence of COVID-19-specific data, it is reasonable to consider extended-duration thromboprophylaxis with LMWH or a DOAC for at least 2 weeks and up to 6 weeks post-hospital discharge in selected COVID-19 patients who are at low risk for bleeding and with key VTE risk factors such as advanced age, stay in the ICU, cancer, a prior history of VTE, thrombophilia, severe immobility, an elevated D-dimer (>2 times ULN), and an IMPROVE VTE score of 4 or more.

Table 8. Prophylactic and therapeutic venous thromboembolism anticoagulation dosing guidelines, modified from the Massachusetts General Hospital.

	Prophylactic Dose	Therapeutic Dose
Unfractionated Heparin (UFH)	5000U Subcut Q12h	80u/kg bolus then 18U/kg/h infusion
Enoxaparin	40mg Subcut Q24h	1mg/kg Subcut Q12h
Fondaparinux	2.5mg Subcut Q24h	5mg Subcut Q24h (<50kg) 7.5mg Subcut Q24h (<50kg-100kg) 10mg Subcut Q24h (>100kg)
Apixaban	2.5mg PO Q12h	
Rivaroxaban	10mg PO Q24h	

25. VTE Treatment in Hospitalized COVID-19 Patients

There are multiple validated and approved strategies to treat hospitalized patients with a new VTE including the use of UFH/LMWH bridging therapy to dose-adjusted warfarin, the use of UFH/LMWH lead-in therapy with a switch to dabigatran/edoxaban, or a monotherapy approach with rivaroxaban/apixaban (214). In hospitalized COVID-19 patients, parenteral anticoagulation with UFH or LMWH may have advantages over other strategies due to the absence of known drug-drug interactions with antiviral agents or investigational therapies used to treat COVID-19. Moreover, the use of LMWH may have further advantages in this setting due to lack of routine monitoring and decrease healthcare worker exposure to infection due to frequent blood draws necessary with IV UFH, which may require higher than usual doses from possible heparin resistance due to acute phase reactants. DOACs may also have further disadvantages in this setting due to potential drug-drug interactions via CYP3A4 mechanisms with certain antivirals (i.e., lopinavir/ritonavir) and immunomodulatory investigational COVID-19 therapies, as well as potential for lack of reversal agents or specific antidotes in some hospitals (16),(122). However, in the post-hospital discharge setting, DOACs provide advantages over vitamin K antagonists such as warfarin due to the lack of the need for routine monitoring and subsequent minimization of patient contact with the healthcare environment.

26. Concluding remarks

Hematological abnormalities are not rare in COVID-19 patients including lymphopenia, neutrophilia, thrombocytopenia, and decline of hemoglobin. When the disease progresses to severe stage, lymphopenia continues to aggravate. Increased neutrophil count and neutrophil-to-lymphocyte ratio, and decreased hemoglobin concentration were identified as the risk factors of severe illness in patients with SARS-CoV-2 infection. The activation of monocyte-macrophage system aggravates the immune damage of the lung and other tissues, which leads to the increase of D-dimer, prothrombin time, and platelet consumption. The hypercoagulable state in COVID-19 is emerging as a major pathological occurrence with serious consequences in mortality and morbidity. The various mechanisms of thrombosis, hematological aspects of the disease and imperativeness of anti-coagulation prophylaxis in COVID-19 patients including those with pre-existing bleeding disorders, Three mechanisms of thrombocytopenia are hypothesized in this review: 1) Direct infection of bone marrow cells by the virus and inhibition of platelet synthesis. 2) Platelet destruction by the immune system.3) Platelet aggregation in the lungs, resulting in microthrombi and platelet consumption; Patients who develop bleeding episodes should be managed according to standard DIC guidelines. Further investigation of the mechanisms of thrombocytopenia can provide a valuable theoretical basis for timely clinical treatment and provide us with a more comprehensive understanding of this disease. Accumulating evidence supports the notion that the hypercoagulability of SARS-CoV-2 involves a unique mechanism of thrombo-inflammation triggered by viral infection, originating in the pulmonary vasculature. Careful evaluation of laboratory indices at baseline and during the disease course can assist clinicians in formulating a tailored treatment approach and promptly provide intensive care to those who are in greater need. Preventive measures for thrombo- prophylaxis and early identification of potentially lethal complications including DIC in order to effectively intervene will improve patient outcomes, and will probably reduce the death rate overall and among infected patients without significant comorbidities.

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