ARTÍCULO DE REVISIÓN

Gac Méd Caracas 2020;128(Supl 1):S3-S16
DOI: 10.47307/GMC.2020.128.s1.2

COVID-19 and associated coagulopathy

Drs. Aixa Müller[1], Andrés Soyano[2]

SUMMARY

COVID-19 is a viral respiratory disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) which presents a varied clinical spectrum. In moderate and severe cases, it can be associated with a coagulopathy that can progress to thrombotic complications and potentially fatal disseminated intravascular coagulation (DIC). In a first phase (moderate disease), coagulopathy manifests with an increase in fibrinogen and D-Dimer and a second phase (severe disease) as a DIC with thrombocytopenia, prolonged prothrombin time (PT) and PTT, decrease in fibrinogen, and marked increase in D-Dimer. Based on the above, thromboprophylaxis with LMWH or unfractionated heparin is recommended during hospitalization and its continuation in the convalescent period at home.

Key words: *COVID-19, coagulopathy, disseminated intravascular coagulation, anticoagulation, D-dimer, fibrinogen, thrombosis.*

RESUMEN

El COVID-19 es una enfermedad respiratoria causada por el coronavirus 2 del síndrome respiratorio agudo severo (SARS-CoV-2), la cual se presenta con un espectro clínico variado. En los casos moderados y graves puede asociarse con una coagulopatía que puede progresar hacia complicaciones trombóticas y coagulación vascular diseminada (CID) potencialmente fatal. En una primera fase (enfermedad moderada) la coagulopatía se manifiesta con aumento del fibrinógeno y del dímero D y en una segunda fase (enfermedad grave) como una CID con trombocitopenia, alargamiento del tiempo de protrombina y del tiempo parcial de protrombina, disminución del fibrinógeno y aumento marcado del dímero D. Con base en lo anterior se recomienda la tromboprofilaxis con heparina de bajo peso molecular o heparina no fraccionada durante la hospitalización y su continuación en el período de convalecencia en casa.

Palabras clave: *COVID-19, coagulopatía, coagulación intravascular diseminada, anticoagulación, dímero D, fibrinógeno, trombosis.*

DOI: https://doi.org/10.47307/GMC.2020.128.s1.2

[1]Hematologist; Chief, El Ávila Clinic Blood Bank, Caracas, Venezuela; Full Professor, Luis Razetti School of Medicine, Central University of Venezuela (UCV); National Academy of Medicine, Venezuela, Chair # 1. ORCID 0000-0001-8421-4441 (asoyano@gmail.com).

[2]Investigador Asociado Titular, Center for Experimental Medicine, Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela; National Academy of Medicine, Venezuela, Corresponding member # 47. ORCID 0000-0002-0728-9295. (soyanolop@gmail.com).

Abbreviations

ACE2: angiotensin-converting enzyme 2; ACE2R: angiotensin-converting enzyme 2 receptor; ARDS: acute respiratory distress syndrome; CRP: C-reactive protein; DD: D-dimer; DIC: disseminated intravascular coagulation; E: envelope; FDP: fibrin degradation product; G-CSF: granulocyte-colony stimulating factor; GM-CSF: granulocyte-macrophage-colony stimulating factor; HCII: heparin

cofactor II; HIF: Hypoxia-inducible factor; ICU: intensive care unit; IFN-γ: interferon-gamma; IL: interleukin; INR: international normalized ratio; ISTH: International Society of Thrombosis and Haemostasis; LDH: lactic dehydrogenase; LMWH: low molecular weight heparin; M: membrane; MCP-1: monocyte chemoattractant protein 1; M-CSF: macrophage colony-stimulating factor; MERS: the Middle East respiratory syndrome; MIP 1-α : macrophage inflammatory protein 1-α; NET: neutrophil extracellular trap; NV: normal values; PAD: peptidyl arginine deiminase; PAR: protease-activated receptor; PCR: polymerase chain reaction; PT: prothrombin time; PTT: partial thromboplastin time; RDS: respiratory distress syndrome; S: spike; SARS: severe acute respiratory syndrome; SIC: sepsis-induced coagulopathy; TNFα: tumor necrosis factor-alpha; UFH: unfractionated heparin; VTE: venous thromboembolism; VWF: von Willebrand factor.

INTRODUCTION

COVID-19 is a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which presents with a varied clinical spectrum. It is usually associated with coagulopathy in moderate and severe cases which might progress to life-threatening thrombotic complications. The disease emerged in December 2019 in Wuhan, China, and quickly spread to most of the world (1). On September 5, 2020, the number of global confirmed cases and global deaths registered by the Johns Hopkins University was 25 683 612 and 875 9433 respectively (global mortality: 3.28 % of confirmed cases), corresponding to Venezuela 50 973 confirmed cases and 412 deaths (0.81 %) (2). A virus was identified in January 2020 from bronchoalveolar lavage samples from 4 patients with atypical pneumonia in Wuhan and its genome was quickly sequenced. This allowed the development of diagnostic tests based on RT-PCR and the detection of viral antigens and their corresponding antibodies (1).

The SARS-CoV-2 (severe acute respiratory syndrome-coronavirus-2)

The causative agent of COVID-19, called SARS-CoV-2, is a virus belonging to the Coronaviridae family. Its genome is made up of a single strand of RNA surrounded by an envelope. The virus contains four primary structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. The spike protein is responsible for binding to host receptors. The M protein contains domains that encapsulate the genome in virions. Protein E is involved in the assembly of the virus. Protein N packages and encapsulates the genome into virions. SARS-CoV-2 binds to a protein receptor on the host cell membrane, the angiotensin-converting enzyme 2 (ACE2), a metallopeptidase (129 KDa) found in cells of the lung, heart, kidney, and intestine (3-6). Once the virus binds to the ACE2 receptor (ACE2R) on the host cell, the viral and cell membranes fuse favoring the entry of viral RNA into the cell (7).

SARS-CoV-2 infection usually begins in the upper respiratory tract from exposure to the virus in aerosol droplets or on contaminated objects (8). The epithelial cells of the oro-nasopharyngeal area and other areas of the respiratory tract have a substantial expression of the ACE-2R, which explains their susceptibility to viral entry (9,10). ACE-2R is also present in other organs such as the stomach, small intestine, spleen, liver, and brain. The density of ACE-2R is particularly high in the lungs, heart, and the endothelium of veins and arteries. High expression of ACE-2R was found in endothelial cells of arteries, small and large veins, and brain arterial smooth muscle cells obtained from different biopsy tissues. ACE-2R mRNA is present in all major organs, although receptor expression is higher in several key organs and locations that play important roles in the onset of infection and its pathophysiological manifestation, including venous, arterial, and microvascular thrombosis (6).

Clinical manifestations and laboratory studies in COVID-19

Several common symptoms have been reported at the onset of the disease: fever (98 %-99 %), dry cough (59 %-76 %), sputum production (27 %-

28 %), fatigue (44 %-70 %), anorexia (40 %), respiratory distress (31 %-55 %), myalgia (35 %), and others of less frequency: nausea, vomiting, lethargy, arthralgia, headache, anosmia, skin rashes, and diarrhea. Some infected people may be asymptomatic, while others may experience acute respiratory distress syndrome (ARDS) and death. Men are affected more frequently (~60 % of cases) with a mean age of approximately 50 years. In 79 patients with COVID-19 hospitalized during July and August 2020 in a private hospital in Caracas, Venezuela (Clínica El Ávila), 80 % were male. Patients with mild symptoms show fever, dry cough, tiredness, mild abnormalities on chest tomography and they have a good prognosis (11,12). In contrast, patients with severe disease develop severe pneumonia, ARDS, or multiple organ failure, with mortality rates ranging between 4.3 % and 15 % according to different authors. It was reported that 138 patients with COVID-19 presented: 1) Epidemiological history, 2) Fever or other respiratory symptoms, 3) Abnormal tomography chest image typical of viral pneumonia, and 4) Positive result of PCR for SARS-CoV-2; of those 138 patients, 13 critically ill met at least one of the following conditions: 1) Dyspnea with respiratory rate ≥30 times/min, 2) Oxygen saturation (resting state) ≤93 %, or 3), PaO2/FiO2 ≤300 mmHg (13). Of the 79 patients hospitalized in Clínica El Ávila, 29 (36.7 %) were considered critically ill and treated in the ICU (Chirinos et al., personal communication).

Although the majority of COVID-19 patients have a mild illness, the study reported by Guan et al. indicates that 16 % of the patients show a more severe disease. The incubation period is 2 to 14 days after exposure to the virus. The risk of severe illness and death in COVID-19 cases increases with age and with the presence of comorbidities, such as heart, lung, kidney, or liver disease, diabetes, immunodeficiency, and obesity (body mass index >40) (14). Among a cohort of 799 patients at Wuhan Tongji Hospital in Wuhan, China, Chen et al. studied 113 who died and 161 who recovered (15). The most frequent symptoms at the onset of the disease were fever and cough. Fever occurred in 92 % of deceased patients and 90 % of recovered patients, and cough in 70 % of deceased patients and 66 % of recovered patients. Other prevalent symptoms at the onset of the disease in the patients who died included fatigue, dyspnea, chest tightness, and sputum production. Dyspnea was more frequent in deceased patients 62 % than in recovered 49 %; chest tightness was presented in 31 % of the deceased vs 30 % recovered. The most frequent complications observed in deceased patients included acute respiratory distress (100 %), respiratory failure (51 %), sepsis (100 %), acute heart problems (77 %), heart failure (49 %), alkalosis (40 %), hypercalcemia (37 %), acute kidney injury (25 %), and hypoxic encephalopathy (20 %). Patients with cardiovascular comorbidity were more likely to develop cardiac complications; regardless of previous cardiovascular disease, acute cardiac injury, and heart failure were more common in deceased patients (15).

Many critically ill COVID-19 patients had typical clinical manifestations of shock, such as cold extremities and a weak peripheral pulse, even in the absence of overt hypotension. In some patients liver and kidney failure and severe lung injury were observed, consistent with the diagnosis of sepsis and septic shock according to the International Sepsis-3 Consensus (16-19). The SARS-CoV-2 infection seemed to be the only cause in most of them. Blood and lower respiratory tract cultures were negative for bacteria and fungi in 76 % of cases. This suggests that viral sepsis was responsible for the clinical manifestations in severe cases of COVID-19. At Clínica El Ávila in Caracas, 55.1 % of the patients admitted at the ICU died (16 out of 29; 11 of them (37.9 %) had septic shock) (Chirinos et al., personal communication). Higher levels of fibrinogen, D-dimer, total bilirubin, transaminases, LDH, creatine kinase, C-reactive protein (CRP), and ferritin have been found in severe patients than in patients with moderate disease (16-19).

Huang et al. reported leukopenia ($<4\times10^9$/L in 10 out of 40 patients [25 %]), significant and sustained lymphocytopenia ($<1.0\times10^9$/L) associated with neutrophilia in mild cases of COVID-19, as well as a significant decrease in CD8+ T cells and increased blood levels of IL-2, IL-6, IL-10, G-CSF (granulocyte-colony stimulating factor), IP10 (the chemokine secreted by cells stimulated with IFN-γ), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1-α (MIP-1 α), and tumor necrosis factor-alpha (TNFα) (11). The

normalization of these values was delayed in patients with severe disease as compared with moderate COVID-19 patients. In this study, the ratio of neutrophils to lymphocytes, the number of CD8 + T cells, and the level of inflammatory cytokines were identified as important prognostic factors for severe COVID-19. Numerous studies have described abnormal levels of the following cytokines and chemokines in patients with COVID-19: IL-2, IL-7, IL-10, macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), 10 kD interferon gamma-induced protein, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-α (MIP 1-α) and tumor necrosis factor-alpha (TNF-α) (11,17,20). Significant increases in interferon-gamma (IFN-γ) in the severe group were only seen 4–6 days after disease onset. Respiratory failure is the cause of death in 70 % of fatal COVID-19 cases. Additionally, uncontrolled release of cytokines in response to viral infection or secondary infections ("cytokine storm") can lead to symptoms of sepsis which is the cause of death in 28 % of severe COVID-19 cases. In these cases, uncontrolled inflammation inflicts damage to multiple organs leading to organ failure, especially the heart, liver, and kidney (10,17). Lymphopenia, leukocytosis, and increased transaminases were associated with poor prognosis. Baseline lymphocyte count was significantly higher in survivors than in non-survivors; in survivors, lymphocyte count was diminished by day 7 after disease onset and improved during hospitalization, whereas permanently marked lymphopenia was observed in those that did not survive. Levels of D-Dimer, high-sensitivity cardiac troponin I, serum ferritin, LDH, and IL-6 were elevated in non-survivors and increased with disease progression. In those who died, high-sensitivity cardiac troponin I increased rapidly from day 16 after disease onset, while LDH increased in both survivors and those who died early in the disease, but in survivors decreased from day 13 (10). Liu et al. observed a sustained decrease in the CD3+, CD8+, and CD4+ T cell counts in patients with severe disease when compared to those with moderate disease. The lowest count was observed 4-6 days after the onset of the disease. They highlight that after the proinflammatory phase with cytokine release there is a sustained and substantial reduction in peripheral lymphocytes, mainly CD4+ and CD8+ T cells, and this reduction indicates poor prognosis (21).

Hyperferritinemia was observed in the severe form of COVID-19. This also occurs in the so-called "hyperferritinemia syndromes", including macrophage activation syndrome, adult-onset Still's disease, antiphospholipid syndrome, and septic shock. These conditions are characterized by elevated serum ferritin and systemic inflammation sustained by a cytokine storm that eventually leads to multi-organ failure (22). Evidence has accumulated in the last decade to support the idea that hyperferritinemia may not only reflect an acute phase response but also play a critical role in inflammation (23).

Thrombosis findings in pathological studies in COVID-19

In a study of the lung tissue of 38 patients who died from COVID-19 at the Luigi Sacco Hospital in Milan and the Papa Giovanni XXIII Hospital in Bergamo (Italy), Carsana et al. found that the predominant pattern was diffuse alveolar damage, similar to that described in severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). Hyaline membrane formation and atypical pneumocyte hyperplasia were common. Platelet and fibrin thrombi were observed in small arteries compatible with coagulopathy in 33 autopsies; as a result of this finding, anticoagulation began to be used in the treatment of COVID-19 (24).

In Hamburg, Germany, Wichman et al. reported 12 autopsies of COVID-19 patients. Deep vein thrombosis, not suspected *ante mortem*, was found in 7 (58 %). Massive pulmonary embolism was the direct cause of death in 4 patients with thrombi derived from the deep veins of the lower extremities; in 3 cases, deep vein thrombosis was present without pulmonary embolism (25). In another study, also in Hamburg, Edler et al. found thrombi in the deep veins of the lower extremities in 32 cases (40 %) in 80 autopsies. There were arterial thrombi in 17 cases (21 %), with 8 cases of fatal pulmonary artery embolisms and 9 cases of peripheral pulmonary artery embolisms. Fifteen of the 80 men who underwent autopsies showed thrombi in the prostatic venous plexus

and one case showed thrombi in the veins of the esophagus (26).

Coagulopathy associated with COVID-19. Abnormal coagulation parameters

Published data indicates that the incidence of thrombosis ranges from 16 % to 49 % in COVID-19 patients admitted to the ICU (10,11,13). In a study of 191 patients (135 from Jinyintan Hospital and 56 from Wuhan Pulmonary Hospital), 50 % of those who died developed coagulopathy as compared to 7 % of the survivors. D-dimer levels greater than 1000 μg/L was associated with a fatal outcome (10).

D-dimer is the main fibrin degradation product. Its presence in the circulation indicates the breakdown of fibrin polymers by plasmin. It is made up of two fibrin D monomers or fragments cross-linked by factor XIII and is generated in the final step of thrombus formation. The amount of circulating D-dimer correlates with the formation of thrombi and with the fibrin load that subsequently undergoes lysis (27).

Alterations in various coagulation parameters have been detected in several studies: D-dimer, prothrombin time, platelet count, fibrinogen, fibrin degradation products (FDP), antithrombin, Factor VIII and von Willebrand Factor (28-30). In the early phase of the disease, there are moderately elevated levels of D-Dimer and FDP, fibrinogen, and platelet count, suggesting adaptive coagulation activity in response to infection and virus-induced inflammation (31). In a study of 5 700 hospitalized COVID-19 patients from the New York area, Richardson et al. reported an average D-dimer level of 438 ng/mL, ranging from 262 to 872 ng/mL (NV: 0–229 ng/mL) (32). As the disease progresses, D-Dimer rises, prothrombin time lengthens and platelet count decreases, and this is associated with more severe disease and higher mortality. Similar results were reported by Guan et al. (14) and also by a multicenter meta-analysis of 30 studies that included 53 000 patients (31). Elevated D-dimer, prolonged prothrombin time, and advanced age are associated with a higher mortality rate at 28 days of illness (33). In a study of 21 deaths from COVID-19, 15 (71 %) met the criteria for Disseminated Intravascular Coagulation (DIC) as a consequence of viral infection, cytokine storm, and organ failure, while DIC developed only in 0.6 % of survivors (34). DIC has been reported in COVID-19 pneumonia generally as a preterminal event (28).

In 150 patients with ARDS treated in 4 ICU of 2 French centers, 64 (46 %) presented thrombotic complications, mainly pulmonary embolisms (16.7 %), clots in the circuit during renal dialysis (96.6 %), and thrombotic occlusion in the centrifuge pump (8 %). The von Willebrand antigen and its activity were increased and 87.7 % developed the lupus anticoagulant. Comparing patients with respiratory failure due to COVID-19 (77 patients) with others without COVID-19 (145 patients), it was found that the former developed more thrombotic complications, mainly pulmonary embolisms (11.7 vs. 2.1 %, $P < 0.008$) (35).

Mechanisms of coagulopathy associated with COVID-19

Four mechanisms have been postulated to explain coagulopathy associated with COVID-19: 1. Endothelial dysfunction. 2. Hypoxia. 3. Presence of viral RNA, and 4. NETosis.

1. Endothelial dysfunction. It has been estimated that in a human of average size there are from 1 to 6 x 10^{13} endothelial cells (a surface area of 4 000-7 000 m^2 and a weight of 1 kg) (36). In the non-activated state, these cells express anticoagulant, antiadhesive, and vasodilator activity, while once activated they express procoagulant, pro adhesive, and vasoconstrictor activity. Tissue Factor, a transmembrane protein, is considered the main initiator of coagulation through its binding to Factor VIIa (activated). *Ex vivo* observations suggest that the endothelial cell is involved in the activation of coagulation mediated by Tissue Factor during severe infection, as occurs in COVID-19 (37). The cytokine-stimulated endothelial cell also expresses a protease-activated receptor (PAR-1) that binds thrombin, Factor IXa (activated), and Factor Xa and increases the expression of Tissue Factor thereby amplifying the coagulation process (38). Thrombin generation is regulated by a set of physiological

coagulation inhibitors and the endothelial cell participates in three systems: a) serine protease inhibitors, such as antithrombin (AT) and heparin cofactor II (HCII), b) the Protein C system, made up of Protein C, Protein S and thrombomodulin, and c) the Tissue Factor Inhibitor pathway. It can be concluded that endothelial cell dysfunction induced by SARS-CoV-2 leads to excess thrombin generation and inhibition of fibrinolysis and therefore thrombosis (39). Von Willebrand Factor (VWF) is synthesized and released mainly by the endothelial cell, although it is also synthesized by megakaryocytes and platelets (40). Interestingly, endothelial cells express ACE2R, the receptor for SARS-CoV-2, which possibly favors endothelial activation by the virus and massive release of VWF leading to the formation of microvascular thrombi. Exocytosis of unusually large VWF multimers initiates microthrombogenesis. These excess multimers anchor to endothelial cells as elongated chains and recruit activated platelets to form "microthrombi", which trigger disseminated intravascular microthrombosis, the underlying pathology of microthrombotic disease associated with endothelial dysfunction or endotheliopathy (40,41).

2. Hypoxia stimulates thrombosis not only through increased blood viscosity by reduced blood flow but also through an increase in the production of Hypoxia-inducible Transcription Factor (HIF). It has been postulated that hypoxia and HIF activation may be a cause and also a consequence of thrombosis in patients with sepsis. Although microthrombosis reduces microvascular blood flow causing local hypoxia and tissue ischemia, sepsis-induced increases in HIF activation could, conversely, increase the expression of clotting factors and integrins that promote thrombus formation (42-44). Chang described the pathogenesis of disseminated intravascular microthrombosis and introduces a "unifying two-way theory" of hemostatic disorders. According to this theory, "normal" hemostasis is triggered by the simultaneous but independent activation of Tissue Factor and "unusually large VWF multimers", while sepsis-associated endotheliopathy is triggered by the activation of unusually large VWF multimers (41). Chang stated that disseminated intravascular coagulation has been inappropriately conceptualized as a fibrin clot disease produced through the Tissue Factor/Factor VIIa-initiated coagulation/coagulation cascade (41). Regardless of the terminology used for microthrombus formation or the environment in which thrombogenesis occurs, thrombus formation is a complex process involving endothelial activation, integrin-mediated platelet-platelet, and platelet-neutrophil aggregation, and cross-linked fibrin formation (45).

3. Presence of viral RNA. Nakazawa et al. demonstrated that circulating RNA could initiate coagulation by serving as a cofactor for the autoactivation of the Factor VII activating protease (46). Kannemeier et al. found that circulating RNA activated contact phase coagulation proteases, such as Factors XI and XII, which exhibited strong RNA binding. On the other hand, it was shown that the administration of RNA to rabbits elicited a significant procoagulant response (47). An experimental model of arterial thrombosis was also designed in mice, in which a vascular lesion was caused with ferric chloride ($FeCl_3$); it was found that extracellular RNA was associated with occlusive thrombi rich in fibrin and pretreatment with RNase (but not DNase) significantly delayed thrombus formation in damaged or necrotic mouse cells. Therefore, under conditions characterized by tissue injury, extracellular RNA serves as a template for thrombosis dependent on contact activation. Thus, the SARS-CoV-2 RNA would initiate coagulation by acting as a cofactor of Factor VII activating protease and the activation of proteases of the coagulation contact system, including Factors XI and XII (47).

4. NETosis o release of white cells nuclear material. Neutrophils, and to lesser extent mast cells, monocytes, and eosinophils, release their nuclear material in response to strong microbial stimulation, pro-inflammatory agents, reactive oxygen species, or activated platelets, forming an extracellular network or trap (NET, neutrophil extracellular trap). The NET formation is a gradual process characterized by the dissolution of the nuclear membrane, decondensation of chromatin, and

cytolysis. These extracellular nets are formed by strands of chromatin wrapped around histones (nucleosomes), citrullination by the enzyme peptidyl arginine deiminase (PAD)-4, chromatin decondensed and interwoven with fibrin strands, broken nuclear membranes, and granules of neutrophils. They are involved in antimicrobial defense. This process is known as NETosis. NETs are an ideal base or template to bind activated platelets, erythrocytes, and leukocytes; they also activate Factor XI and generate thrombin for fibrin formation. There is also a vital or non-lytic NETosis, in which nuclear materials (DNA and histones) are released without rupture of the cell membrane (48-53). Histone citrullination is a hallmark of chromatin decondensation in neutrophils; it is the conversion of arginine to citrulline (an unconventional amino acid) mediated by PAD-4, an enzyme expressed in granulocytes. Histone hypercitrullination is detected in highly decondensed chromatin in granulocytes and blood neutrophils of HL-60 (an experimental cellular line). PAD-4 inhibition decreases histone hypercitrullination and the formation of NET-like structures, while PAD-4 treatment of HL-60 cells facilitates these processes (54-56). NETs are part of a sterile inflammation with thrombosis that can involve all vascular beds, including the microvascular circulation (57,58).

Venous and Arterial Thrombosis in COVID-19

Klok et al. evaluated the incidence of arterial and venous thrombotic complications in 184 critically ill patients with COVID-19 and pneumonia hospitalized in the ICU (59). The complications detected were pulmonary embolism, deep vein thrombosis, ischemic stroke, myocardial infarction, or systemic arterial embolism. 31 % of the patients had thrombotic complications, mainly venous, the most common being pulmonary embolism; 13 % of the patients (23 of them) died. The independent predictors of thrombosis were: increased incidence of thrombosis with advanced age and coagulopathy (lengthening of prothrombin time greater than 3 seconds or above the upper limit and PTT more than 5 seconds). All patients received nadroparin thromboprophylaxis. None of the patients who experienced thrombotic events met strict criteria for DIC. Therefore, the authors recommend the use of high thromboprophylactic doses of low molecular weight heparin (LMWH) in these patients.

Tang et al. retrospectively evaluated the efficacy of anticoagulation in COVID-19 patients by studying 449 patients with severe disease (defined as respiratory rate >30 breaths/min, oxygen saturation <93 %, PaO2/FiO2 <300 mmHg), with coagulopathy (60); 99 patients received LMWH for 7 days or more. In a multivariate analysis, elevated D-dimer, prolonged prothrombin time, and advanced age were positively correlated with mortality at 28 days, while platelet number was negatively correlated with mortality in the same period. No differences were found in mortality at 28 days between the patients treated with or without heparin (30.3 % vs 29.7 %, P=0.910). But mortality at 28 days of heparin users was lower than that of non-users in patients with sepsis-induced coagulopathy (SIC) with SIC score ≥4 (40.0 % vs 64.2 %, P=0.029), or with a D-Dimer >6 times the upper limit of normality (32.8 % vs 52.4 %, P=0.017). They concluded that anticoagulant therapy (LMWH x 7 days or more) is associated with a better prognosis in patients with severe COVID-19 who meet the criteria for SIC (score ≥ 4) or with D-Dimer >6 times higher than the normal limit (60).

Helms et al. studied 150 patients with ARDS admitted to the ICU (35). The risks of thromboembolic disease in patients suffering from COVID-19 were compared with patients who had Respiratory Distress Syndrome (RDS) associated with another cause. Pulmonary embolisms occurred in 16.7 % of COVID-19 patients and only in 2.1 % of non-COVID-19 patients. Of the patients who required dialysis, 29 (96.6 %) presented clots in the circuit; 2 patients, out of 12 who required extracorporeal membrane oxygenation due to refractory hypoxemia, presented thrombotic occlusions in the circuit. The authors did not find patients with DIC but reported a marked increase in antigen and the activity of VWF and Factor VIII. Lupus anticoagulant was detected in 50 out of 57 patients (35).

Chen et al. studied 99 hospitalized patients

in Wuhan and found 6 % with prolonged aPTT, 5 % with prolonged prothrombin time, 36 % with elevated D-dimer, and increased biomarkers of inflammation, including interleukin-6 (IL-6), erythrocyte sedimentation rate, and C-reactive protein. Thrombocytopenia occurred in only 12 % (16). On the other hand, also in Wuhan, Wang et al. found minimal elevations in prothrombin time and normal aPTT in 138 patients (13).

The initial phase of COVID-19-associated coagulopathy is characterized by a prominent elevation of D-Dimer and FDP, whereas abnormalities in prothrombin time, PTT, and platelet count are relatively rare in that phase. Coagulation abnormalities are observed at the onset of SARS-CoV-2 infection, but no clinical bleeding occurs. There is significant inflammation in SARS-CoV-2 infected patients with increased levels of IL-6, CRP, erythrocyte sedimentation rate, and fibrinogen (16,61). Infectious complications in critically ill patients activate systemic coagulation and inflammatory responses, vital for host defense but which can lead to DIC (62,63). Patients with severe infection may have coagulopathy associated with DIC, according to the criteria of the International Society of Thrombosis and Haemostasis (ISTH), and intense activation of the coagulation cascade and consumption of its factors. This induces thrombocytopenia (platelet count <50 x 109/L), prolongation of prothrombin time and aPTT, marked increase in D-Dimer, and decrease in fibrinogen (<1.0 g/L) (64). The ISTH not only has diagnostic criteria for DIC but also developed and validated a SIC score (65-67).

Microorganisms and their components induce the expression of numerous immune factors, including tissue factor in monocytes and macrophages, by binding to pattern-recognizing receptors on immune cells (68,69). The triggering of inflammatory reactions by the host also results in increased production of pro-inflammatory cytokines that have pleiotropic effects, including activation of coagulation, which, if left unchecked, can lead to consumption coagulopathy.

Guidelines for the treatment of coagulopathy or DIC

Coagulopathy or DIC associated with COVID-19 rarely causes bleeding even with abnormal coagulation parameters. Ranucci et al. demonstrated that aggressive thromboprophylaxis could decrease fibrinogen and D-Dimer levels and thrombotic events that occur in patients admitted to the ICU (70). Elevated D-Dimer (> 6 times the upper normal limit) was found to have decreased mortality when treated with prophylactic doses of enoxaparin or unfractionated heparin (33). Since COVID-19 infection is complicated by extensive thrombosis that requires anticoagulant and/or thrombolytic treatment, several organizations have designed therapeutic guidelines to treat this aspect of the disease; for example, the American Society of Hematology recommends pharmacological thromboprophylaxis with LMWH. This treatment is preferable to unfractionated heparin unless the patient is considered to have an increased risk of bleeding compared to thrombosis. If there is a history of heparin-induced thrombocytopenia, fondaparinux, a pentasaccharide chemically related to LMWH which inhibits Factor Xa, can be used as an alternative. It should be considered to adjust the dose of anticoagulants in obese patients; the recommended dose is 40 mg BID. If LMWH is contraindicated due to renal failure (creatinine clearance <30 mL/min), unfractionated heparin (UFH) can be used as an alternative and follow the dosage guide (71).

In patients in which anticoagulants are contraindicated, devices for pneumatic compression of the legs should be used. Prophylactic doses of LMWH are recommended for all COVID-19 hospitalized patients even with abnormal coagulation parameters in the absence of active bleeding; this treatment should be suspended only if the platelet count is $<25x10^9$/L or fibrinogen <0.5 g/L. Thus, prolonged prothrombin time or activated PTT is not a contraindication for anticoagulant treatment.

The combined use of thromboprophylaxis and pneumatic compression devices is not recommended. It is currently unknown whether critically ill COVID-19 patients should receive therapeutic anticoagulation in the absence of confirmed or suspected venous thromboembolism (VTE). Instead of the empirical use of heparin in therapeutic doses in patients with COVID-19 without another indication for anticoagulation, participation in clinical protocols is encouraged.

In the case of patients who experience recurrent clotting of access devices such as central venous catheters, arterial lines, or extracorporeal circuits (e.g., continuous renal replacement therapy, extracorporeal membrane oxygenation), despite prophylactic anticoagulation, it might be reasonable to increase the intensity of anticoagulation (e.g., from the standard intensity prophylaxis to intermediate intensity prophylaxis or from intermediate intensity prophylaxis to therapeutic intensity) or switching anticoagulants in these settings. Risk factors should be considered, including age, comorbidities such as active cancer and elevated D-dimer> 2 times the upper normal limit. Currently, an elevation in D-Dimer is not an indication to augment the anticoagulation dose.

In critically ill hospitalized patients treatment with LMWH or UFH is preferred to direct oral anticoagulants due to its shorter half-life and fewer drug-drug interactions. Patients treated regularly with warfarin who cannot control INR during isolation may be candidates for direct oral anticoagulant therapy, but patients with mechanical heart valves, ventricular assist devices, valvular atrial fibrillation, antiphospholipid antibody syndrome should generally continue warfarin treatment. Considering that UFH, LMWH, and warfarin do not accumulate in breast milk and do not induce an anticoagulant effect in the newborn, breastfeeding women treated regularly with anticoagulant must continue this therapy. LMWH and UFH remain the anticoagulants of choice during pregnancy. Any decision to use post-discharge thromboprophylaxis should consider each patient's risk factors for the venous thromboembolic disease at discharge, including reduced mobility and risk of bleeding, as well as viability. Post-discharge thromboprophylaxis should be performed with direct oral anticoagulants, either betrixaban (160 mg as the first dose, followed by 80 mg daily for 35-42 days) or rivaroxaban (10 mg daily for 31-39 days) (71). Similar guidelines are followed by the Canadian Critical Care Society and the Association of Medical Microbiology and Infectious Disease of Canada (72), the British Haematology Society (73), and the Italian Society on Thrombosis and Haemostasis (74). The British Haematology Society has recommended the use of the DIC-ISTH score as a prognostic indicator of the treatment guide for patients with COVID-19; specifically in the absence of a bleeding phenotype therapeutic doses of anticoagulants should be considered although prophylactic doses of UFH or LMWH are recommended (73). ISTH highlights in its guide of the management of COVID-19 coagulopathy that the increase in D-dimer, absence of thrombocytopenia, and late-onset DIC is indicative of poor prognosis in some patients, for which it also recommends the use of LMWH at a prophylactic dose. The recommendation is the use of prophylactic doses of LMWH depending on the appearance of additional data (75).

The Spanish Society of Cardiology recommends prescribing LMWH to all hospitalized patients, with weight-adjusted doses for those with a body mass index >35 and after assessing the bleeding risk and the baseline platelet count. Discharged patients go into a convalescent-phase that can increase thromboembolic events and mortality due to the reduction of mobility that it entails; for this reason, it is recommended to prolong the use of LMWH in prophylactic doses for 7-10 days after discharge (76).

The American College of Cardiology has recommended pharmacological prophylaxis of VTE in patients with COVID-19 who require ICU care, as well as in those with pneumonia, respiratory failure, or other comorbidity factors such as heart failure, cancer, prolonged periods of immobility, and pregnant women who are hospitalized. Prolonged prophylaxis after discharge was considered reasonable for high-risk patients (reduced mobility, comorbidity factors such as active cancer, and an elevated D-dimer at discharge) Anticoagulation should be continued for 6 weeks for catheter-associated thrombosis and at least 3 months for VTE (77).

Wang et al. carried out a trial with the natural tissue plasminogen activator (tPA) in three COVID-19 patients with severe ARDS on a ventilator and treatment with heparin (78). Transient improvement in lung function expressed by the Kirby index (the ratio between the increases in arterial oxygen pressure/fraction of inspired oxygen PAO_2/FiO_2) was observed in 2 out of 3 patients together with a reduction in fibrinogen (3/3) after two sequential bolus doses of 25 mg intravenous infusion of tissue

plasminogen activator (tPA) but without bleeding complications. It remains to be determined whether a larger bolus (50-100 mg) or a new dose can achieve a more sustained response. Certainly, emergent arterial occlusions (i.e., myocardial infarction and ischemic stroke) require more aggressive thrombolytic therapy with careful evaluation of factors that may increase the risk of bleeding (78,79).

There are insufficient data related to the use of other anticoagulants, including thrombin inhibitors (dabigatran), Factor Xa inhibitors (fondaparinux, rivaroxaban, apixaban, edoxaban, betrixaban), or PAR-1 (protease-activated receptors) inhibitors (a novel class of antiplatelet agents) as antithrombotic prophylaxis of COVID-19.

Also, there are insufficient data related to the treatment of thrombosis in COVID-19 with the use of intravenous recombinant tissue plasminogen activator. The role of thrombolytic or fibrinolytic agents in the treatment of ARDS and thrombotic complications associated with COVID-19 are not yet elucidated. Streptokinase treatment appears to be a salvage rescue therapy for severe adult ARDS that can improve oxygenation and lung mechanics faster than heparin or conventional management (33,34,77).

The use of blood components is reserved for active bleeding, which is not frequent in COVID-19. On the contrary, bleeding is rare in the context of COVID-19. If the patient presents bleeding, the same principles indicated in the ISTH guidelines for the management of DIC related to blood transfusions should be followed. Platelet concentrate should be used only if the platelet count is $<50x10^9$/L, fresh frozen plasma if International Normalized Ratio or INR is >1.8, and 10 Units of cryoprecipitate if fibrinogen is ógeno 1.5 g/L. If there is severe coagulopathy and bleeding due to liver dysfunction, prothrombin complex concentrate should be used instead of plasma since blood volume appears to be a significant factor associated with respiratory compromise (80,81).

CONCLUSIONS

Published data indicates that the incidence of thrombosis ranges from 16 % to 49 % in COVID-19 patients admitted to the ICU. Clinical research with worldwide groups of patients led to the concept that COVID-19 can manifest clinically as a coagulopathy in the early phase of the disease with moderately elevated levels of D-Dimer and FDP, fibrinogen, and platelet count (coagulopathy associated with COVID-19) and in severe cases DIC with thrombocytopenia, lengthening of time prothrombin (PT) and PTT, marked increase in D-Dimer and decrease in fibrinogen, and this is associated with higher mortality, suggesting adaptive coagulation activity in response to infection and virus-induced inflammation. Elevated D-dimer, prolonged prothrombin time, and advanced age are associated with a higher mortality rate at 28 days of illness Disseminated Intravascular Coagulation (DIC) as a consequence of viral infection, cytokine storm, and organ failure. According to the results expressed, numerous Medical Societies or Working Groups recommend thromboprophylaxis with LMWH or unfractionated heparin during hospitalization and its continuation in the period of convalescence at home. It is emphasized that all hospitalized patients with COVID-19 should receive LMWH. For COVID-19 patients receiving routine anticoagulation for other indications (recent diagnosis of VTE, atrial fibrillation, mechanical heart valves, or secondary prevention of VTE), this should be continued. In critically ill hospitalized patients treatment with LMWH or UFH is preferred to direct oral anticoagulants due to its shorter half-life and fewer drug-drug interactions.

Acknowledgment: We thank Dr. L. Chirinos et al. for sharing their data on COVID-19 patients treated at Clínica El Ávila, Caracas, Venezuela.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727-733.
2. Johns Hopkins University of Medicine Coronavirus Resource Center. 2020. https://coronavirus.jhu.edu/.
3. Becker RC. Toward understanding the 2019 Coronavirus and its impact on the heart. J Thromb

Thrombolysis. 2020;50(1):33-42.

4. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS coronavirus. J Virol. 2020;94(7):e00127-20.
5. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;46(4):586-590.
6. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203(2):631-637.
7. Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M, et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. J Virol. 2007;81(16):8722-8729.
8. Bar-On YM, Flamholz A, Phillips R, Milo R. SARS-CoV-2 (COVID-19) by the numbers. eLife. 2020;9: e57309.
9. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12(1):8.
10. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet. 2020;395(10229):1054-1062.
11. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
12. Chan JF, Yuan S, Kok KH. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. Lancet. 2020;395(10223):514-523.
13. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-1069.
14. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-1720.
15. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. Br Med J. 2020;368: m1091.
16. Chen N, Zhou M, Dong X, Qu J, Gong F, Hang Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet. 2020;395 (10223):507-513.
17. Zhang B, Zhou X, Qiu Y, Feng F, Feng J, Feng J, et al. Clinical characteristics of 82 death cases with COVID-19. *medRxiv* 2020.02.26.20028191.
18. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-810.
19. Lin GL, McGinley JP, Drysdale SB, Pollard AJ. Epidemiology and immune pathogenesis of viral sepsis. Front Immunol. 2018;9:2147.
20. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev. 2020;S1359-6101(20)30109-X.
21. Liu J, Li S, Liu J, Liang B, Wang X, Wang H. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. Ebio Medicine. 2020;55:102763.
22. Colafrancesco S, Alessandri C, Conti F, Priori R. COVID-19 gone bad: A new character in the spectrum of the hyperferritinemic syndrome?. Autoimmun Rev. 2020;19(7):102573.
23. Recalcati SP, Invernizzi P, Arosio G. Cairo. New functions for an iron storage protein: The role of ferritin in immunity and autoimmunity. J Autoimmun. 2008;30(1-2):84-89.
24. Carsana L, Sonzogni A, Nasr A, Rossi R, Pellegrinelli A, Zerbi P, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: A two-centre descriptive study. Lancet Infect Dis. 2020:S1473-3099(20)30434-5.
25. Wichmann D, Sperhake J-P, Lütgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: A Prospective Cohort Study. Ann Intern Med. 2020;173(4):268-277.
26. Edler C, Aepfelbacher M, FitzekA, Heinemann A, Heinrich F. Dying with SARS-CoV-2 infection - An autopsy study of the first consecutive 80 cases in Hamburg, Germany. Int J Legal Med. 2020;134(4):1275-1284.
27. Becker R. COVID-19 update: COVID-19-associated coagulopathy. J Thromb Thrombolysis. 2020;15:1-14.
28. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-847.
29. Colantuoni A, Martini R, Caprari P, Ballestri M, Capechi PL, Gnasso A, et al. COVID-19 Sepsis and Microcirculation Dysfunction. Front Physiol.

2020;11:747.

30. Fogarty H, Townsend L, Cheallaigh CN, Bergin C, Martin-Loeches I, Browne P, et al. COVID-19 Coagulopathy in Caucasian patients. Br J Haematol. 2020;189(6):1044-1049.
31. Ma C, Gu J, Hou P, Zhang L, Bai Y, Guo Z, et al. Incidence, clinical characteristics and prognostic factor of patients with COVID-19: A systematic review and meta-analysis. medRxiv 2020.03.17.20037572.
32. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5 700 patients hospitalized with COVID-19 in the New York City Area. JAMA. 2020;323(20):2052-2059.
33. Miesbach W, Makris M. COVID-19: Coagulopathy, Risk of Thrombosis, and the Rationale for Anticoagulation. Clin Appl Thromb Hemost. 2020;26:1076029620938149.
34. Fei Y, Tang N, Liu H, Cao W. Coagulation dysfunction: A hallmark in COVID-19. Arch Pathol Lab Med. 2020;10.5858.
35. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: A multicenter prospective cohort study. Intensive Care Med. 2020;46(6):1089-1098.
36. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood. 1998;91(10):3527-3561.
37. Solovey AA, Gui L, Solovey AN, Harkness J, Hebbel RP. Modulation of endothelial cell activation in sickle cell disease: A pilot study. Blood. 2001;97:1937-1941.
38. Jaffe EA, Hoyer LW, Nachman RL. Synthesis of von Willebrand factor by cultured human endothelial cells. Proc Natl Acad Sci USA. 1974;71(5):1906-1909.
39. Huntington JA. Natural inhibitors of thrombin. Thromb Haemost. 2014;111(4):583-589.
40. Escher R, Breakey N, Lämmle B. Severe COVID-19 infection associated with endothelial activation. Thromb Res. 2020;190:62. DOI:10.1016/j.thromres. 2020.04.014.
41. Chang JC. Sepsis and septic shock: endothelial molecular pathogenesis associated with vascular microthrombotic disease. Thromb J. 2019;30(17):10.
42. Evans CE. Hypoxia and HIF activation as a possible link between sepsis and thrombosis. Thromb J. 2019; 14(17):16.
43. Dempfle CE. Coagulopathy of sepsis. Thromb Haemost. 2004;91:213-224.
44. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood. 2003;101:3765–3777.
45. Peters K, Unger RE, Brunner J, Kirkpatrick CJ. Molecular basis of endothelial dysfunction in sepsis. Cardiovasc Res. 2003;60:49-57.
46. Nakazawa F, Kannemeier C, Shibamiya A, Song Y, Tzima E, Schubert U, et al. Extracellular RNA is a natural cofactor for the (auto-)activation of Factor VII-activating protease (FSAP). Biochem J. 2005;385(Pt 3):831-838.
47. Kannemeier C, Shibamiya A, Nakazawa F, Trusheim H, Ruppert C, Markart P, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. Proc Natl Acad Sci USA. 2007;104(15):6388-6393.
48. von Köckritz-Blickwede M, Goldmann O, Thulin P, Heinemann K, Norrby-Teglund A, Rohde M, et al. Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. Blood. 2008;111(6):3070-3080.
49. Chow OA, von Köckritz-Blickwede M, Bright AT, Hensler ME, Zinkernagel AS, Cogen AL, et al. Statins enhance formation of phagocyte extracellular traps. Cell Host Microbe. 2010;8(5):445-454.
50. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, et al. Neutrophil extracellular traps promote deep vein thrombosis in mice. J Thromb Haemost. 2012;10(1):136-144.
51. Brinkmann V, Zychlinsky A. Beneficial suicide: Why neutrophils die to make NETs. Nat Rev Microbiol. 2007;5(8):577-582.
52. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303(5663):1532-1535.
53. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007;176(2):231-241.
54. Thålin C, Daleskog M, Göransson SP, Schatzberg D, Lasselin J, Laska AC, et al. Validation of an enzyme-linked immunosorbent assay for the quantification of citrullinated histone H3 as a marker for neutrophil extracellular traps in human plasma. Immunol Res. 2017;65(3):706-712.
55. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol. 2009;184(2):205-213.
56. Nakashima K, Hagiwara T, Yamada M. Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. J Biol Chem. 2002;277(51):49562-49568.
57. Gould TJ, Vu TT, Swystun LL, Dwivedi DJ, Mai SH, Weitz JI, et al. Neutrophil extracellular traps promote thrombin generation through platelet-dependent

and platelet-independent mechanisms. Arterioscler Thromb Vasc Biol. 2014;34:1977-1984.

58. Semeraro F, Ammollo CT, Morrissey JH, Dale GL, Friese P, Esmon NL, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: Involvement of platelet TLR2 and TLR4. Blood. 2011;118(7):1952-1961.
59. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145-147.
60. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost. 2020;18:1094-1099.
61. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. Blood. 2020;135(23):2033-2040.
62. Iba T, Levy JH, Thachil J, Wada H, Levi M. Scientific and Standardization Committee on DIC of the International Society on Thrombosis and Haemostasis. The progression from coagulopathy to disseminated intravascular coagulation in representative underlying diseases. Thromb Res. 2019;179:11-14.
63. Iba T, Levy JH, Wada H, Thachil J, Warkentin TE, Levi M. Subcommittee on Disseminated Intravascular Coagulation. Differential diagnoses for sepsis-induced disseminated intravascular coagulation: Communication from the SSC of the ISTH. J Thromb Haemost. 2019;17(2):415-419.
64. Bakhtiari K, Meijers JCM, de Jonge E, Levi M. Prospective validation of the International Society of Thrombosis and Haemostasis scoring system for disseminated intravascular coagulation. Crit Care Med. 2004;32(12):2416-2421.
65. Suzuki K, Wada H, Imai H, Iba T, Thachil J, Toh CH, Subcommittee on Disseminated Intravascular Coagulation. A re-evaluation of the D-dimer cut-off value for making a diagnosis according to the ISTH overt-DIC diagnostic criteria: Communication from the SSC of the ISTH. J Thromb Haemost. 2018;16(7):1442-1444.
66. Iba T, Levy JH, Warkentin TE, Thachil J, van der Poll T, Levi M. Scientific and Standardization Committee on DIC, and the Scientific and Standardization Committee on Perioperative and Critical Care of the International Society on Thrombosis and Haemostasis. Diagnosis and management of sepsis-induced coagulopathy and disseminated intravascular coagulation. J Thromb Haemost. 2019;17(11):1989-1994.
67. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M. Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost. 2001;86(5):1327-1330.
68. Woei-A-Jin FJ, van der Starre WE, Tesselaar ME, García Rodríguez P, van Nieuwkoop C, Bertina RM, et al. Procoagulant tissue factor activity on microparticles is associated with disease severity and bacteremia in febrile urinary tract infections. Thromb Res. 2014;133(5):799-803.
69. Iba T, Levy JH. Inflammation and thrombosis: Roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. J Thromb Haemost. 2018;16(2):231-241.
70. Ranucci M, Ballotta A, Di Dedda U, Bayshnikova E, Dei Poli M, Resta M, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. J Thromb Haemost. 2020;18(7):1747-1751.
71. American Society of Hematology. COVID-19 and VTE/Anticoagulation: Frequently Asked Questions. 2020. https://www.hematology.org/covid-19/covid-19-and-vte-anticoagulation.
72. Canadian Critical Care Society and Association of Medical Microbiology and Infectious Disease (AMMI) Canada. Clinical management of patients with moderate to severe COVID-19 - Interim guidance. https://www.canada.ca/en/public-health/ services/ diseases/2019-novel-coronavirus-infection/clinical-mangement-covid-19.html.
73. BSH. Haemostasis and Thrombosis Task Force. Disseminated Intravascular Coagulation. https//b-s-h.org.uk, 2020.
74. Marietta M, Ageno W, Artoni A, De Candia E, Gresele P, Marchetti M, et al. COVID-19 and haemostasis: A position paper from Italian Society on Thrombosis and Haemostasis (SISET). Blood Transfus. 2020. DOI: 10.2450/2020.0083-20.
75. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Hemost. 2020;18(5):1023-1026.
76. Vivas D, Roldán V, Esteve-Pastor MA, Roldán I, Tello-Montoliu A, Ruiz-Nodar, et al. Recomendaciones sobre el tratamiento antitrombótico durante la pandemia COVID-19. Posicionamiento del Grupo de Trabajo de Trombosis Cardiovascular de la Sociedad Española de Cardiología [Recommendations on antithrombotic treatment during the COVID-19 pandemic. Position statement of the Working Group on Cardiovascular Thrombosis of the Spanish Society of Cardiology]. Rev Esp Cardiol. 2020;73(9):749-757.
77. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for

Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(23):2950-2973.

78. Wang J, Hajizadeh N, Moore EE, McIntire RC, Moore PK, Veress LA, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A case series. 2020;18(7):1752-1755.
79. Huang LT, Chou HC, Wang LF and Chen CM. Tissue plasminogen activator attenuates ventilator-induced lung injury in rats. Acta Pharmacol Sin. 2012;33(8):991-997.
80. Wada H, Thachil J, Di Nisio M, Mathew P, Kurosawa S, Gando S, et al. Guidance for diagnosis and treatment of DIC from harmonization of the recommendations from three guidelines. The Scientific Standardization Committee on DIC of the International Society on Thrombosis Haemostasis. J Thromb Haemost. 2013; 10.1111/jth.12155. DOI: 10.1111/jth.12155.
81. Wada H, Matsumoto T, Yamashita Y. Diagnosis and treatment of disseminated intravascular coagulation (DIC) according to four DIC guidelines. J Intensive Care. 2014;2(1):15.