

Coagulopathy in COVID-19

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Abstract

Hemostatic disorders play an important role in the pathogenesis and clinical manifestations of COVID-19. The purpose of the research was a detailed consideration of the pathogenesis, clinical manifestations, and methods of diagnosing and treatment of coronavirus-induced coagulopathy (CIC). At the onset of COVID-19, hypercoagulability is detected, and consumption coagulopathy and disseminated intravascular coagulation (DIC) syndrome are usually observed at later stages of the disease. In the pathogenesis of hypercoagulation in patients with COVID-19, pro-inflammatory cytokines, hyperfibrinogenemia, increased blood levels of von Willebrand factor, factor VIII, neutrophilic extracellular traps, platelet activation, production of antiphospholipid antibodies, microvesicles are of importance. Laboratory findings show increased plasma concentrations of D-dimer, fibrinogen, a longer prothrombin time and a decrease in the number of platelets. The cumulative incidence of thrombotic complications ranges from 21 to 31%. Thrombosis risk factors are intensive care unit stay, leukocytosis, and a high plasma D-dimer concentration. Differential diagnosing of CIC should be carried out with disseminated intravascular coagulation, sepsis-induced coagulopathy, antiphospholipid, hemophagocytic syndromes, thrombotic microangiopathy, and heparin-induced thrombocytopenia. CIC may be complicated by sepsis, antiphospholipid syndrome, hemophagocytic syndrome, thrombotic microangiopathy, and heparin-induced thrombocytopenia.

The main therapy is low molecular weight heparins treatment. Treatment recommendations are provided.

Key words: coronavirus infection 2019, heparin, coagulation, D-dimer, fibrinogen, prothrombin time.

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Коагулопатия при COVID-19

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Резюме

Нарушения гемостаза играют важную роль в патогенезе и клинических проявлениях COVID-19. Целью работы явилось подробное рассмотрение патогенеза, клинических проявлений, методов диагностики и лечения коронавирус-индуцированной коагулопатии (КИК). При дебюте COVID-19 выявляется гиперкоагуляция, а коагулопатия потребления, синдром диссеминированного внутрисосудистого свертывания (ДВС) регистрируются обычно на поздних стадиях заболевания. В патогенезе гиперкоагуляции при COVID-19 играют роль провоспалительные цитокины, гиперфибриногенемия, повышенное содержание в крови фактора Виллебранда, фактора VIII, нейтрофильных внеклеточных ловушек, активация тромбоцитов, выработка антифосфолипидных антител, микровезикулы. В лабораторных показателях выявляются повышенные плазменные концентрации D-димера, фибриногена, увеличение протромбинового времени и уменьшение количества тромбоцитов. Кумулятивная частота тромботических осложнений колеблется от 21 до 31 %. Факторами риска тромбозов являются пребывание в отделении интенсивной терапии, лейкоцитоз и высокая концентрация D-димера в плазме. Дифференциальный диагноз КИК следует проводить с ДВС-синдромом, сепсис-индуцированной коагулопатией, антифосфолипидным, гемофагоцитарным синдромами, тромботической микроангиопатией, гепарин-индуцированной тромбоцитопенией. Возможно сочетание КИК с сепсисом, антифосфолипидным синдромом, гемофагоцитарным синдромом, тромботической микроангиопатией, гепарин-индуцированной тромбоцитопенией.

Основной терапией является лечение низкомолекулярными гепаринами. Приводятся рекомендации по лечению.

Ключевые слова: коронавирусная инфекция 2019, гепарин, коагуляция, D-димер, фибриноген, протромбиновое время.

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The novel COroNaVirus Disease 2019 (COVID-19) is an infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped RNA recombinant virus (genus Betacoronavirus) between the bat coronavirus and an origin-unknown coronavirus. The genetic sequence of SARS-CoV-2 is up to 79% similar to that of SARS-CoV [1, 2]. The first outbreak of this infection was reported in the Chinese city of Wuhan in December 2019, and as early as March 11 2020 the World Health Organisation (WHO) declared this out-

break as a pandemic. The world entered a “war to the knife”, as was underscored by Italian specialists [3], which is not figurative, but literal. The infection starts when SARS-CoV-2 enters target cells expressing angiotensin-converting enzyme-2 (ACE2), a membrane receptor. ACE2 receptors are expressed in respiratory, renal, and cardiac cells as well as in the oesophagus, bladder, ileum, and central nervous system. However, type II alveolar cells are the major target, which is rapidly reached by the virus.

Viral infection of these cells leads to diffuse alveolar damage, which clinically manifests as acute respiratory distress syndrome (ARDS). This syndrome has been reported in 41.8% of patients, half of which died [4]. Nevertheless, if we continue comparing the fight with COVID-19 to a war, we can say that it is at least a two-front war: lung damage is the most evident clinical manifestation of COVID-19, but there is a second, often “invisible”, front in this war, i.e. coagulation abnormalities, which are frequently inapparent and left undetected. Coagulopathies not only lead to clinically significant thrombotic events, but are also implicated in the pathogenesis of coronavirus infection, including lung injury. Alteration in microcirculation caused by microthrombi may significantly worsen acute respiratory failure in patients with COVID-19. Therefore, treatment protocols for COVID-19 must include therapies for haemostatic disorders. Evaluation of mechanisms underlying coronavirus-induced coagulopathy (CIC) helps not to only better understand the pathogenesis of the disease, but also improves the accuracy of its diagnosis and opens up new horizons for treatment.

The objective of this paper is to describe the pathogenesis and clinical manifestations of CIC and discuss methods used to detect and treat this condition.

Pathogenesis of coronavirus-induced coagulopathy

Cell penetration of SARS-CoV-2 is mediated by its spike (S) protein, which binds to the receptor on the surface of the host cell. This receptor is ACE2 on the surface of type II alveolar cells. Single-cell RNA-sequencing revealed that expression of the ACE2 gene is limited to a small population of type II alveolar cells and that endothelial cells and alveolar macrophages do not have ACE2 [5]. Receptor-mediated endocytosis results in release of the viral nucleocapsid into the cytosol, where the viral RNA serves as an mRNA for the synthesis of the pp1a and pp1ab polypeptides, of which, in the next replication/translation passage, a copy of the virus RNA is formed, as well as 8 separate mRNA templates for virus proteins that generate them indefinitely [6]. Released cytokines provoke interstitial inflammation, endothelial damage, and blood coagulation activation. Tissue factor is crucial in the pathogenesis of blood coagulation activation. It is exposed by monocytes and by damaged endothelial cells or those activated by the cytokines' burden.

The final result is thrombin production and consequent thrombosis of alveolar capillaries [7]. Although inflammation and coagulation are the key factors in the pathogenesis of CIC, the mechanisms underlying coagulation disturbances are different from those involved in disseminated intravascular coagulation (DIC) and sepsis-induced coagulopathy (SIC) [8].

The procoagulant response in sepsis involves damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) [8]. Sepsis is associated with release of proinflammatory cytokines such as interleukin (IL)-1 β , IL-6, tumor necrosis factor, and complement system proteins, all of which induce coagu-

lopathy [9]. In addition, tissue factor expression on monocytes and macrophages, neutrophil activation, and release of neutrophil extracellular traps (NETs) also produce activation of coagulation and thrombosis [10, 11]. NETosis is a type of programmed death of neutrophils. During this process necrotic neutrophils release a mixture of filaments mainly consisting of DNA, histone proteins, and nucleosomes, which also exhibit procoagulant activity and increase the risk of thrombosis. Such thromboinflammation leads to endothelial damage and an increased generation of thrombin [12]. SIC/DIC is associated with inhibition of fibrinolysis caused by an increased production of plasminogen activator inhibitor 1, which is accompanied by thrombosis and organ dysfunction [13]. To detect these abnormalities, the following parameters were included in the DIC criteria: severity of thrombocytopenia, elevated plasma levels of fibrin degradation products, including D-dimer, increased prothrombin time, and hypofibrinogenemia [14]. The following parameters are the SIC criteria: increased prothrombin time, severity of thrombocytopenia, and severity of the patient's condition, as assessed by the Sequential Organ Failure Assessment (SOFA) [15]. Both DIC and SIC are associated with a reduced plasma activity of natural anticoagulants (antithrombin III and protein C) [16].

CIC occurs through other mechanisms. Several research groups have reported that coagulation profiles of patients with COVID-19 reflect severe hypercoagulation, but not consumptive coagulopathy or DIC [17, 18]. Consumptive coagulopathy is a typical manifestation of DIC/SIC, but it does not develop at the onset of COVID-19. In COVID-19 interleukin-1 β and interleukin-6 may cause thrombocytosis and hyperfibrinogenemia in patients with SARS-CoV-2-associated ARDS [19]. In early stages of COVID-19, inflammation and hypercoagulation are mainly observed in the lungs and DIC develops only if the diseases progress to multiorgan dysfunction. Significantly elevated plasma levels of D-dimer are explained by impaired regulation of local alveolar fibrinolysis via urokinase-type plasminogen activator released from alveolar macrophages. These events lead to viral interstitial pneumonia, ARDS, and death of patients with coronavirus infection [19]. SARS-CoV-2 invasion of endothelial cells, which widely express the ACE2 receptor (receptor for SARS-CoV-2), results in massive release of plasminogen activator [20]. In healthy conditions, ACE2 enhances anticoagulant properties of vascular endothelium. SARS-CoV-2 binding to ACE2 induces expression of tissue factor and inhibits the protein C system [20].

As the severity of COVID-19 increases, procoagulant activity leads, over time, to fibrin generation, an increase in blood fibrinogen levels, and platelet activation. Damaged endothelial cells release von Willebrand factor and plasminogen activator inhibitor 1, which causes increased clot formation in pulmonary capillaries. Plasma activity of factor VIII, an acute-phase protein, is three times higher and concentrations of von Willebrand factor antigen are five times higher than those in healthy subjects [18].

As in sepsis, NETs have proven to be involved in COVID-19. Analysis of blood samples obtained from 50 patients with COVID-19 and 30 healthy subjects

showed that patients with coronavirus infection had higher levels of the following key markers of NETosis: cell-free DNA, myeloperoxidase (MPO)-DNA complexes, and citrullinated histone H3. Elevated blood levels of cell-free DNA associated with higher platelet levels. Plasma from individuals with COVID-19 added to neutrophils triggered NETosis of these cells *in vitro* [21]. Through electrostatic interactions, neutrophil extracellular traps activate the intrinsic (contact) phase of coagulation [22]. As compared with controls, patients with COVID-19 and thrombosis had higher blood levels of markers of NETs. Levels of these markers were evaluated in a retrospective, case-control study of 11 patients with COVID-19 who developed thrombosis as compared with gender- and age-matched COVID-19 patients without clinical thrombosis [23]. Compared to the control group, patients with thrombosis had significantly higher blood levels of markers of NETs (cell-free DNA, MPO-DNA complexes, and citrullinated histone H3). There was a strong association between markers of NETs and D-dimer levels.

Production of antiphospholipid antibodies is another mechanism of involvement of haemostasis in the pathogenesis of COVID-19. Chinese authors [24] reported three cases of COVID-19 patients who developed thrombotic events (arterial thrombosis in the limbs and ischaemic stroke) on days 33, 10, and 18 from disease onset. More detailed testing showed the presence of anti- β_2 -glycoprotein I IgA and IgG antibodies as well as anticardiolipin IgA antibodies. French researchers [25] studied 56 patients with verified COVID-19 and reported that 25 (45%) of them had lupus anticoagulant, whereas anticardiolipin and anti- β_2 -glycoprotein I antibodies were detected in only five of 50 tested patients (10%). In another study 50 (87.7%) of 57 tested COVID-19 patients had positive lupus anticoagulant [26]. In COVID-19 the insidiousness of lupus anticoagulant is that, on the one hand, patients have increased activated partial thromboplastin times (APTT) and, on the other, suffer from thrombotic events. Moreover, APTT cannot guide therapeutic decisions regarding heparin therapy in lupus anticoagulant-positive patients.

Some authors [18] focus considerable attention on the role of microvesicles in the development of hypercoagulation in COVID-19. These moieties are cytoplasmic microparticles stemming from platelets or monocytes and exhibit procoagulant properties.

Change in haemostatic parameters in coronavirus-induced coagulopathy

Apparently, a consideration of the pathogenic pathways is needed to understand what happens in CIC. In the pandemic environment, however, clinicians treating patients with COVID-19 need simple diagnostic criteria that would make it possible to detect CIC and choose appropriate therapy.

Plasma levels of D-dimer. The degree of increase in blood levels of D-dimer reflects the severity of patient's condition. A study of data regarding 1,099 patients with laboratory-confirmed COVID-19 from more than 550 hospitals in China showed that 260 (46.4%) out

of 560 patients had plasma levels of D-dimer higher than 0.5 mg/L. Of note, elevated D-dimer levels were observed only in 43% of patients with mild disease and in 60% of patients with severe disease [27]. C. Huang *et al.* [28] noted that at the time of presentation patients with COVID-19 who required admission to an intensive care unit (ICU) had higher plasma D-dimer levels than those who did not: median D-dimer level 2.4 mg/L (0.6 – 14.4 mg/L) vs 0.5 mg/L (0.3 – 0.8 mg/L), $p = 0.0042$). N. Tang *et al.* [29] showed that higher plasma D-dimer levels in patients with COVID-19 were a predictor of death: mean D-dimer level 2.12 $\mu\text{g/mL}$ (interquartile range 0.77 – 5.27 $\mu\text{g/mL}$) in non-survivors vs 0.61 $\mu\text{g/mL}$ (interquartile range 0.35 – 1.29 $\mu\text{g/mL}$) in survivors ($p < 0.001$) (normal value $< 0.50 \mu\text{g/mL}$). Fibrin degradation product (FDP) levels, another marker of activation of coagulation and fibrinolysis, had the same predictive value. The mean FDP level was 7.6 $\mu\text{g/mL}$ (interquartile range 4.0 – 23.4 $\mu\text{g/mL}$) in non-survivors vs 4 $\mu\text{g/mL}$ (interquartile range 4.0 – 4.3 $\mu\text{g/mL}$) in survivors ($p < 0.001$) (normal value $< 5.0 \mu\text{g/mL}$). Italian authors [30], who measured plasma D-dimer levels in 388 patients with COVID-19 every three days (on days 1 – 3, 4 – 6, and 7 – 9 after admission), reported a tendency toward elevation of this parameter in more severe patients, which was more evident in patients transferred to an ICU. These levels were always higher in non-survivors than in survivors, indicating greater severity of their condition. In patients who were not transferred to an ICU elevation of D-dimer was not very high (median D-dimer level increased from 329 to 472 ng/mL in survivors and from 868 to 1,093 ng/mL in non-survivors), while in ICU patients these values were of different magnitude (median level increased from 615 to 3,137 ng/mL in survivors and from 1,022 to 7,746 ng/mL in non-survivors). N. Tang *et al.* [29] reported that patients had significantly high plasma D-dimer and FDP levels in the first days of COVID-19, but starting from day 10 this difference became especially pronounced. In a study of 201 patients with COVID-19, Coxregression analysis showed that elevated plasma D-dimer levels were a risk factor associated with the development of ARDS (hazard ratio [HR], 1.03; 95% confidence interval [CI], 1.01 – 1.04), $p < 0.001$ and death (HR, 1.02; 95% CI, 1.01 – 1.04), $p < 0.002$ [4]. In a study of 343 patients, ROC analysis showed that plasma D-dimer concentrations of $\geq 2.0 \mu\text{g/mL}$ on admission in hospital, i.e. 4 times higher than normal, predicted in-hospital mortality with a sensitivity of 92.3% and a specificity of 83% [31]. Elevated plasma levels of D-dimer are not, however, pathognomonic only for COVID-19: comparison of haemostatic parameters of 449 patients with SARS-CoV-2 pneumonia and acute respiratory failure and those of 104 patients with pneumonia of the same severity and respiratory failure caused by other etiologic agents did not show any significant difference in their D-dimer levels [32].

APTT. This parameter does not significantly change in COVID-19. Nevertheless, prolonged APTT in patients with COVID-19 may be caused, as mentioned above, by the presence of lupus anticoagulant. It did not significantly differ between COVID-19 patients who required ICU treatment and those who did not (26.2 [22.5 –

33.9] s vs 27.7 [24.8 – 34.1] s, $p = 0.57$) [33] as well as between survivors and non survivors (41.2 [36.9 – 44.0] s vs 44.8 [40.2 – 51.0] s, $p = 0.096$) [29]. APTT was not a significant risk factor for ARDS (HR, 0.97 [95% CI: 0.94 – 1.01], $p = 0.13$) or death (HR, 0.96 [95% CI, 0.91 – 1.01], $p = 0.06$) [4].

Prothrombin time. Prolonged prothrombin time is not a disease-specific sign of COVID-19 [26]. This parameter did not significantly differ in patients with pneumonia induced by SARS-CoV-2 and non-SARS-CoV-2 pneumonia [32]. However, prolonged prothrombin time is associated with higher severity of COVID-19 and acts as a risk factor for the development of ARDS: (HR, 1.56 [95% CI, 1.32 – 1.87], $p < 0.001$) [4]. In patients with COVID-19 longer prothrombin times were observed in those who required ICU treatment compared to those who did not (12.2 [11.2 – 13.4] s vs 10.7 [9.8 – 12.1] s, $p = 0.012$) as well as in non-survivors compared to survivors (15.5 [14.4 – 16.3] s vs 13.6 [13.0 – 14.3] s, $p < 0.001$). Of note, although these differences are statistically significant, the absolute difference is just a few seconds, and if INR is measured and prothrombin time is not, these differences may not be noted. A dynamic evaluation revealed prolongation of prothrombin time in non-survivors compared to survivors starting from day 10 after admission [29].

Plasma levels of fibrinogen. While DIC is associated with hypofibrinogenaemia, which is considered one of its diagnostic criteria [14], COVID-19 is more often associated with hyperfibrinogenaemia. In the study of 183 patients with COVID-19 conducted by *N. Tang et al.* [29] the median plasma fibrinogen level was 4.55 g/L (3.676 – 5.17 g/L), with the normal range being 2.0 – 4.0 g/L. Fibrinogen levels did not significantly differ in survivors and non-survivors, while a dynamic assessment showed that on days 10 and 14 from the onset of the disease non-survivors had higher fibrinogen levels than survivors [29]. Fibrinogen is an acute-phase protein, whose concentration increases in inflammation. This can explain a strong correlation ($R_2 = 0.506$) between plasma levels of fibrinogen and IL-6 in COVID-19 patients [34].

Antithrombin III. Unlike in sepsis, in COVID-19 plasma activity of antithrombin III was not reduced and in most patients it remained within the reference range or slightly decreased [18, 26, 29]. In general, activity of antithrombin III did not significantly differ between non-survivors and survivors [29]. However, daily monitoring showed that after the first week of hospital stay non-survivors had lower activity of antithrombin III (still generally within the reference range) than survivors [29]. This could be explained not only by consumption of antithrombin III due to infection, but also by heparin therapy, which itself causes depletion of antithrombin III.

Coagulation factor VIII. This coagulation factor is actually an acute-phase protein, thus in most patients with COVID-19 its plasma activity is 3 – 4 times higher than normal [18, 26].

Von Willebrand factor. In most patients with COVID-19 levels of von Willebrand factor antigen were 4 – 6 times higher than normal [18, 26], which reflected the severity of endothelial damage resulting in its release.

Platelets. At the onset, COVID-19 is typically associated with moderate thrombocytopenia. In a study of 1,099 patients with COVID-19, the median platelet level was $168 \times 10^9/L$. On admission, thrombocytopenia, which was defined as platelet count $< 150 \times 10^9/L$, was present in 36.2% of patients [27]. In pneumonia caused by SARS-CoV-2 thrombocytopenia is less severe than in those of other etiologies [32]. A meta-analysis including nine studies with 1,779 COVID-19 patients [35] showed that severe COVID-19 was associated with more significant thrombocytopenia than mild disease: weighted mean difference (WMD) was $31 \times 10^9/L$, this number shows how much lower the platelet count was. A subgroup analysis comparing patients by survival showed WMD of $48 \times 10^9/L$. Moreover, thrombocytopenia was associated with five-fold enhanced risk of severe disease (odds ratio [OR], 5.1; 95% CI, 1.8 – 14.6) and mortality in patients with COVID-19 [35].

Thus, the following parameters should be measured in all COVID-19 patients: plasma levels of D-dimer, prothrombin time, platelet count, and plasma levels of fibrinogen (specified in descending order of diagnostic value). The frequency of measuring D-dimer and fibrinogen levels, prothrombin time, and platelet count depends on the severity of COVID-19. Both an increase, and a decrease in these parameters are important changes. In hospital, the recommended frequency of measurements is every four-five days for patients with mild disease, every two days for those with moderate disease, and every day for those with severe disease. If the infection worsens, additional unscheduled testing for these parameters should be performed [36]. It can help triage COVID-19 patients by severity. The results of these tests have prognostic value [37].

Another option is integral diagnostic tests for COVID-19-associated haemostatic disorders. Some authors have reported using thromboelastography (TEG) in COVID-19 patients and showed that this method, as well as clotting tests, can detect hypercoagulation [18]. Another useful method is rotation thromboelastometry (ROTEM). The ROTEM panel includes INTEM, EXTEM, and FIBTEM tests which are able to detect hypercoagulation. In non-survivors the signs of hypercoagulation, as assessed by ROTEM tests, were most significant [17]. TEG and ROTEM can also be used to monitor treatment with heparin [38].

Clinical manifestations of coronavirus-induced coagulopathy

Whatever laboratory signs of haemostatic disorders are, what is most important is their clinical manifestations. Among 92 patients with COVID-19 who were admitted to an ICU, forty percent experienced thrombotic events, including venous (79%) and arterial (21%) thrombosis. Nineteen (21%) of these patients experienced haemorrhagic events [39]. Haemorrhagic events in COVID-19 may be caused by a direct effect of the virus, thrombocytopenia and DIC in severe cases, or anticoagulation therapy. In one study [40], these events were observed in 3% of individuals who received anticoagulants and in 1.9% of those who did not receive this treatment ($p = 0.2$).

Different authors report different data about the frequency and incidence of thrombotic events because these parameters are highly dependent on the examinations performed. Undoubtedly, some conditions are difficult to detect, such as pulmonary embolism (PE) in patients with ARDS, asymptomatic deep-vein thrombosis, or ischaemic stroke in patients on mechanical ventilation who are undergoing a medically induced sedation to an unconscious state.

In a study of 184 patients with verified COVID-19, PE was diagnosed in 13.6%, deep-vein thromboses and catheter-related thromboses in 1.6%, and ischaemic stroke in 1.6% of the patients. A cumulative incidence of thromboses was 31% [41]. These authors [41] emphasised that thrombotic events were especially difficult to detect in patients on mechanical ventilation.

In a study of 362 patients with COVID-19 who were treated in an academic hospital in Milan, the rate of thrombotic events was 7.7%, corresponding to a cumulative rate of 21.0%, despite the fact that all patients had received thromboprophylaxis with low-molecular-weight heparin (LMWH) starting from day 1 of hospital stay. A cumulative rate of thromboembolic events was significantly higher for ICU patients (27.6%) compared to that for patients who were treated in general wards (6.6%). Most thrombotic events were diagnosed within 24 h of hospital admission. Overall, PE was detected in 1.2%, deep-vein thromboses in 1.4%, catheter-related deep-vein thromboses in 2.1%, ischaemic cerebral stroke in 2.5%, and acute coronary syndrome in 1.1% of patients. These data were obtained not in the whole population of hospitalised COVID-19 patients but only in those who had undergone relevant examinations that are able to detect the above-mentioned complications. This may explain great differences in the rate of thrombotic events because their detection was highly dependent on the examination protocol adopted in a hospital. In another study, contrast-enhanced computed tomography (CT-angiography) was performed in all 106 patients with COVID-19 and revealed PE in 32 (30%) patients [42]. Other authors performed ultrasound examination in all COVID-19 patients on mechanical ventilation and observed thrombotic events in 22.2% of cases, including deep-vein thromboses in 14.8%, three-fourths of which were catheter-related, and a thrombus attached to the tricuspid valve [43]. Therefore, regular CT-angiography and ultrasonography in all COVID-19 patients receiving hospital treatment will increase detection rates for thrombotic events, and longer hospital stays will be associated with higher rates of these complications. This was confirmed by a study conducted by *S. Middeldorp et al.* who reported that [44] 39 (20%) out of their 198 COVID-19 patients admitted in hospital were diagnosed with thromboembolism, despite thrombosis prophylaxis. The cumulative incidences of thromboses at 7, 14 and 21 days of hospital stay were 16, 33%, and 42%, respectively. For symptomatic thrombotic events, these were 10, 21, and 25%, i.e. almost 1.5 times lower. This once again proves that all patients with COVID-19 should be evaluated for thrombotic events irrespective of the presence of their clinical symptoms. The incidence of thromboses was significantly higher in ICU (26, 47, and

59% at 7, 14 and 21 days) than in general wards (5.8, 9.2, and 9.2% at 7, 14, and 21 days). In addition to ICU stay, leukocytosis and high plasma D-dimer levels were also risk factors for thrombotic events. Patients with thrombotic events had a 2.4-fold increased risk of death (HR, 2.4; 95% CI, 1.02 – 5.5) [44].

French authors compared CT-angiography data in 106 patients with ARDS secondary to COVID-19 and 54 patients with ARDS who did not have COVID-19. Pulmonary embolism was detected in 32 (30%) patients with ARDS secondary to COVID-19 and only in 6 (11%) out of 54 patients with ARDS without COVID-19, i.e. three times less often [42]. Thus, patients with ARDS secondary to COVID-19 develop PE significantly more often than those with ARDS of other etiologies. Eighty-two percent of patients with COVID-19 infection and D-dimer levels of $> 5,000 \mu\text{g/L}$ had pulmonary embolus, while 78% of patients without pulmonary embolus had D-dimer levels $< 5,000 \mu\text{g/L}$ [42].

CT pulmonary perfusion showed that early in the course of COVID-19, when D-dimer levels are below 500 ng/mL , there are multiple bilateral perfusion deficits due to microvascular obstruction. Pulmonary embolism is highly likely to occur at later stages of the disease and should be suspected if the person develops haemoptysis, unexplained tachycardia, or signs/symptoms of deep-vein thrombosis or have D-dimer level above 500 mg/L [45].

Autopsy of 12 patients who died of COVID-19 revealed thrombi in the deep veins of the lower extremities in 7 (58%) patients in whom thrombosis was not detected before death; pulmonary embolism was the direct cause of death in 4 (33%) out of these 12 patients [46].

Differential diagnosis of coronavirus-induced coagulopathy

Foremost, differential diagnosis of CIC should include DIC and septic coagulopathy. Unlike CIC, DIC and septic coagulopathy are more often accompanied by the following signs, which are also more severe in these disorders: thrombocytopenia, consumption of natural anticoagulants (protein C and antithrombin III), hypofibrinogenaemia, increased APTT, and prothrombin time (Table). Does it mean that COVID-19 cannot be accompanied by DIC? Research has shown that, although in most cases the onset of the disease is really marked by non-DIC hypercoagulation disorders, DIC may develop later, as the disease progresses and multiorgan dysfunction, infectious complications or sepsis appear. Thus, while in COVID-19 survivors DIC was reported only in 0.6% of cases, in those who died it was observed already in 71.4% of cases [29].

COVID-19 can be complicated by antiphospholipid syndrome, resulting in the appearance of such additional clinical and laboratory signs as venous and arterial thrombosis and increased APTT (Table).

Haemophagocytic syndrome (HFS) is characterized by hyperactivation of immune cells (macrophages, natural killers, and cytotoxic T-cells) and cytokine storm, which is similar to the pathogenesis of COVID-19 [47, 48]. HFS can be caused by various factors, including viral infections,

Table
Changes in hemostatic parameters in patients with various diseases and syndromes
Таблица
Изменения параметров гемостаза при различных заболеваниях и синдромах

Parameter	CIC	DIC/SIC	APS	HFS	HUS	TTP	HIT II
Thrombosis	Microthrombosis, venous thrombosis	Microthrombosis	Venous/arteri thrombosis	Microthrombosis, venous thrombosis	Microthrombosis, venous/arteri thrombosis	Microthrombosis, venous/arteri thrombosis	Venous/arteri thrombosis
Platelets	→↓	↓↓	↓	↓	↓	↓↓	↓↓
D-dimer	↑↑	↑	↑	→	→	→	↑
APTT	→	↑	↑↑	→			→
PT	↑	↑	→	→			→
Fgn	↑	↓↓	→	→	→	→	→
Antithrombin III	→	↓↓	→	→	→	→	→
APS-antibodies	+	No	++	No	No	No	No
Complement activation	+	No	No	No	+++	No	No
Von Willebrand factor multimers	No	No	No	No	+++	+++	No
Schizocytes	No	No	No	No	+++	+++	No
ADAMTS-13, %	> 10	> 10	> 10	> 10	> 10	< 10	> 10
HIT-antibodies	No	No	No	No	No	No	++

Note: CIC – coronavirus-induced coagulopathy; DIC – disseminated intravascular coagulation; SIC – sepsis-induced coagulopathy; aPL Ab – antiphospholipid antibodies; APS – antiphospholipid syndrome; HFS – haemophagocytic syndrome; HUS – haemolytic uraemic syndrome; TTP – thrombotic thrombocytopenic purpura; HIT – heparin-induced thrombocytopenia; APTT – activated partial thromboplastin time; PT – prothrombin time; Fgn – fibrinogen; AT – antithrombin.

and may have similar clinical manifestations (Table) [47]. In one study 35 patients with COVID-19 were reported to have signs of haemophagocytosis in their bone marrow aspirate samples, two-line cytopenia, and hyperferritinaemia, which misled the authors to interpret their condition as HFS [48]. This led to objections of other researches, who believe that hyperinflammation and hypercytokinaemia in the acute phase of severe COVID-19 infection are not caused by HFS, but reflect the severity of ARDS and lung injury [49].

Atypical haemolytic uraemic syndrome (aHUS) and thrombotic thrombocytopenic purpura (TTP) are thrombotic microangiopathies and constitute a group of conditions with different pathogenesis but a similar clinical presentation of microvasculature damage, nonimmune microangiopathic haemolytic anaemia, consumptive thrombocytopenia, and ischaemic organ injury (Table). aHUS is caused by impaired regulation of the complement system, which leads to its uncontrolled activation [50, 51]. The pathophysiology of TTP involves reduced activity of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif), a plasma protein regulating interaction of von Willebrand factor with platelets.

Inactivity or reduced activity of ADAMTS13 leads to consumption of platelets in developing microthrombi that obstruct arterioles and capillaries and cause intravascular mechanical haemolysis and clinical manifestations of TTP [52]. These conditions are not typically associated with impairments in the plasma haemostasis system; they are defined by the following signs:

- multimers of von Willebrand factor circulating in blood;
- normal plasma levels of von Willebrand factor antigen;
- a blood smear showing a large number of schistocytes due to mechanical haemolysis.

In TTP microthrombi are found most extensively in the heart, brain, kidneys, and pancreas, whereas aHUS most commonly affects kidneys [52]. A group of authors [53] reported five cases of microvascular injury and thrombosis due to complement activation in patients with COVID-19 and respiratory failure. All patients had thrombocytopenia accompanied by such histological characteristics as fibrin deposits within vascular lumens and deposition of terminal complement components C5b-9 (membrane attack complex), C4d, and C3d7 in the skin and lungs, which is similar to HUS. These authors did not report nonimmune

haemolytic anemia or schistocytes in blood. They believe that SARS-CoV-2 affects the proximal complement via the lectin and classical pathways as well as the terminal components of the complement system via inflammation, activation of platelets and endothelial cells, and white blood cell recruitment [54]. Like other researchers, we have never seen schistocytes in blood or haemolysis [54] in our patients, and all of them had plasma activity of ADAMTS13 above 10%.

Type II heparin-induced thrombocytopenia (HIT) (see Table) is caused by antibodies against complexes of heparin and platelet factor 4 containing in platelet α -granules. The resulting complexes consisting of IgG, platelets, and platelet factor 4 bind platelet Fc receptors. This causes platelet activation, aggregation, and destruction, resulting in the release of procoagulant phospholipids [55]. Clinical manifestations of type II HIT appear about 5 – 14 days after the onset of heparin treatment and include thromboses and thrombocytopenia. Type II HIT is diagnosed based on the platelet count $< 100 \times 10^9/L$ or 50% reduction of the platelet count from baseline, which appears between days 5 and 10 after the onset of heparin treatment; the appearance of arterial or venous thromboses; and exclusion of other causes of thrombocytopenia [55]. The incidence of HIT in patients receiving unfractionated heparin (UFH) and those receiving LMWH was 4.8 per thousand patients and 0.48 per thousand patients, respectively [56]. Coagulopathy is not typical for type II HIT. However, as most patients with COVID-19 receive heparin, the risk of type II HIT cannot be fully ruled out. A group of American authors [57] reported that the cumulative incidence of positive HIT immunoassay (positive antibodies to complexes of heparin and platelet factor 4) was 12% at 25 days. For all patients with positive antibodies, heparin treatment was replaced by argatroban.

Thus, CIC should be differentiated from other syndromes and disorders complicated by thrombotic events. It must, however, be kept in mind that, on the one hand, COVID-19 may be associated with other preexisting disorders, such as antiphospholipid syndrome (APS), TTP, etc., and on the other hand, these disorders may complicate the course of COVID-19, for example, lead to the appearance of lupus anticoagulant, type II HIT, sepsis, etc. In such cases CIC is accompanied by other haemostatic disorders.

Treatment of coronavirus-induced coagulopathy

LMWH is the main and widely available treatment for CIC [37]. There are several targets for heparin in CIC. In addition to its anticoagulant properties, heparin exhibits a number of other positive effects:

- in the lungs it reduces inflammation and clot formation, decreases the severity of ARDS, and improves oxygenation;
- in the heart it reduces clot formation in the coronary arteries and cardiac chambers, decreases the severity of cardiomyopathy and cardiac dysfunction caused by ischaemic hypoxia in the subendothelial tissue;

- in other organs it also reduces the severity of microvascular ischaemia, multiorgan dysfunction, the intensity of oedema and capillary leakage.

The anionic nature of heparin allows it to bind to several proteins and thus act as an effective inhibitor of viral attachment [58]. In an experimental model, heparin (100 $\mu g/mL$) reduced the number of infected cells in sputum of patients with SARS-CoV-2 pneumonia by 50% [59]. Moreover, the SARS-CoV-2 S1 protein receptor-binding domain interacts with heparin [60].

According to the International Society on Thrombosis and Haemostasis (ISTH) guidelines, prophylactic dose heparin should be considered in all patients (including non-critically ill) who require hospital admission for COVID-19 infection, in the absence of any contraindications (active bleeding and platelet count less than $25 \times 10^9/L$) [37]. Below is the treatment strategy for CIC based on a summary of guidelines on heparin therapy established by various communities (ISTH [37], Chinese experts [38], the Swiss Society of Hematology [61], and Russian guidelines [36]):

An assessment of haemostatic disorders in patients with severe COVID-19 infection should include medical history (congenital disorders of coagulation, thrombophilia, platelet dysfunction, treatment with anticoagulants or anti-platelet agents, etc.).

Prophylaxis of deep-vein thromboses of lower extremities/PE should be considered in COVID-19 patients who are quarantined and being treated at home if they are at high risk of venous thromboembolic events, but at low risk of haemorrhage and are not receiving anticoagulants for other indications. With regard to this, special attention should be given to patients with limited mobility, a history of thrombotic events, or malignancies, especially those who have additional risk factors of thrombosis.

LMWH, at least in prophylactic doses, should be given to ALL hospitalised patients and not discontinued at least until discharge. There is no evidence showing the superiority of a particular LMWH agent over others. If LMWH is not available or contraindicated, UFH can be used.

Routine monitoring of blood anti-Xa activity in patients receiving parenteral anticoagulants is not required. It can be considered in patients at higher risk of haemorrhage and/or thrombosis. The target levels of anti-Xa activity are 0.2 – 0.6 anti-Xa units/mL for preventive treatment and 0.6 – 1.0 anti-Xa units/mL for therapeutic treatment. In patients receiving LMWH, blood samples for anti-Xa activity are taken between four and six hours after drug administration (preferably after three-four injections), in patients receiving subcutaneous UFH it is done in the intervals between injections, and in those receiving intravenous (IV) infusions of UFH six hours after each dose adjustment.

After discharge prolonged prophylaxis (preferably with LMWH) can be considered for COVID-19 patients if they are still at higher risk of venous thromboembolic events, but at low risk of haemorrhage and do not require therapeutic doses of anticoagulants for other indications.

Contraindications to the use of prophylactic doses of LMWH/UFH include continued bleeding, the platelet

count $< 25 \times 10^9/L$, and severe renal failure (for LMWH). Prolonged prothrombin time and APTT are not contraindications for the use of LMWH/UFH.

Patients with thrombotic events should receive therapeutic doses of LMWH/UFH. Administration of LMWH/UFH in therapeutic doses can also be considered in patients with clinical signs suspicious for thrombotic events when the diagnosis cannot be verified. In ICU patients with significantly elevated plasma levels of D-dimer, severe inflammation, renal or hepatic dysfunction, or respiratory failure should receive therapeutic doses of LMWH/UFH. Possible causes of heparin resistance include high levels of acute-phase proteins (C-reactive protein, fibrinogen, and factor VIII) or von Willebrand factor, low plasma antithrombin III activity, and type II HIT.

In patients with fluctuations in the platelet count and/or heparin resistance, type II HIT should be excluded. In patients with type II HIT, venous thromboembolic events should be prevented and treated with fondaparinux sodium. Unlike LMWH/UFH, fondaparinux sodium does not produce potentially beneficial pleiotropic effects, but it does not cause heparin-induced thrombocytopenia.

LMWH and fondaparinux sodium should not be used in patients with severe renal failure or rapidly changing renal function.

There is no information about the use of direct oral anticoagulants in COVID-19. If patients with mild COVID-19 are receiving oral anticoagulants for other indications, they can continue this treatment. In case of unacceptable drug interactions with medications for COVID-19 (lopinavir/ritonavir) as well as severe COVID-19, patients should be switched to therapeutic doses of heparin (preferably LMWH).

In non-bleeding patients with consumptive coagulopathy, the platelet count should be maintained above $20 \times 10^9/L$ and plasma levels of fibrinogen above 2 g/L. In patients with bleeding, the platelet count should be maintained above $20 \times 10^9/L$, plasma levels of fibrinogen above 2 g/L, and prothrombin ratio below 1.5. Fibrinogen levels of < 1.5 g/L, or FFMA < 10 mm (as measured by TEG), or MCFBITEM ≤ 6 mm are indications for cryoprecipitate therapy. For non-bleeding thrombocytopenic patients, the threshold for platelet transfusion is $20 \times 10^9/L$ and for bleeding patients and those awaiting lumbar puncture it is $50 \times 10^9/L$. If bleeding persists, recombinant activated factor VII can be used.

Patients with a creatinine clearance > 30 mL/min must receive LMWH. For patients with body weight above 100 kg, an increase in dose should be considered in advance.

Patients with a creatinine clearance < 30 mL/min must receive UFH either subcutaneously two or three times a day or as a continuous intravenous infusion.

Anti-Xa activity should be monitored in patients with renal failure who are receiving LMWH.

It is not necessary to monitor plasma antithrombin III activity, but it must be monitored in individual cases (in patients with DIC, sepsis or heparin resistance).

In patients with COVID-19, UFH therapy can be monitored using TEG with heparinase or ROTEM (INTEM and HEPTEM tests). Comparison of data obtained by

TEG and ROTEM with and without heparinase helps evaluate the effectiveness of heparin therapy. The recommended parameters include the ratio of the R time in the control tube (without heparinase) to the R time in the heparinase tube (R/Rh ratio) or ROTEM (CTINTEM/CTHEPTEM ratio) data.

In COVID-19 patients receiving renal replacement therapy, UFH/LMWH should be used for systemic anticoagulation. If UFH is used for anticoagulant therapy, dose titration should be based on TEG (R/Rh ratio) or ROTEM (CT INTEM/CTHEPTEM ratio) data. If LMWH is used, it is administered as an intravenous bolus injection (60 – 80 IU/kg) 20 – 30 minutes before the procedure and additionally at a dose of 30 – 40 IU/kg every 4 – 6 hours. Anti-Xa activity should be maintained at 0.3 IU/mL. Systemic anticoagulation should not be used in patients undergoing citrate dialysis.

Patients undergoing extracorporeal membrane oxygenation should receive UFH with the aim of reaching activated clotting time of 180 – 220 s, or APTT 1.5 times higher than normal, or R/Rh ratio as measured by TEG, or anti-Xa activity of 0.3 – 0.7 IU/mL. Heparin therapy can improve treatment outcomes in COVID-19 patients. Comparison of treatment results in a group of patients ($n = 449$) with COVID-19, only 99 of whom received heparin (mainly LMWH) for 7 days or longer, did not show any difference in 28-day mortality between heparin users and nonusers (30.3% vs 29.7%, $p = 0.910$). However, the heparin treat was associated with lower 28-day mortality in patients with SIC score ≥ 4 (40.0% vs 64.2%, $p = 0.029$) and in patients with D-dimer exceeding 6-fold of upper limit of normal (32.8% vs 52.4%, $p = 0.017$) [62].

The study conducted by *L.Ayerbe et al.* [63] included patients with COVID-19, admitted in 17 hospitals in Spain. Among them, 1,734 people received heparin and 285 did not. Among the heparin users 242 (14.0%) people had died, and among the nonusers 59 (20.7%) patients had died. Heparin was associated with lower mortality when the model was adjusted for age and gender, with OR (95% CI) 0.55 (0.37 – 0.82), $p = 0.003$. This association remained when hypoxaemia ($\text{SaO}_2 < 90\%$), and fever (temperature $> 37^\circ\text{C}$) were added to the model with OR (95% CI), 0.54 (0.36 – 0.82), $p = 0.003$. *I.Paranjpe et al.* [40] conducted a study of 2,773 patients with verified COVID-19, only 786 (28%) of whom received systemic anticoagulation. In-hospital mortality did not significantly differ in patients who were treated with anticoagulants and those who were not (22.5% vs 22.8%). Patients who received anticoagulation were significantly more likely to require mechanical ventilation (29.8% vs 8.1%; $p < 0.001$), which can be explained by more severe disease in this subgroup. In patients who required mechanical ventilation, anticoagulation improved prognosis (mortality was 29.1% with a median survival of 21 days for those treated with anticoagulation as compared to 62.7% with a median survival of 9 days in patients who did not receive anticoagulation (HR, 0.86; 95% CI, 0.82 – 0.89, $p < 0.001$).

Nevertheless, besides systemic heparin therapy, there have been other attempts to treat CIC. A new trial of

inhalation therapy with heparin and N-acetylcysteine (nebulized Heparin-N-acetylcysteine in COVID-19 Patients by Evaluation of pulmonary function, HOPE) is being currently developed. The theoretical rationale for this type of therapy is that the SARS-CoV-2 virus has a Spike Protein that interacts with three molecules on the surface of lung cells: heparin sulfate, furin, an enzyme required for processing, and an ACE2 receptor [64]. These interactions are needed for the virus to infect cells. The combination of heparin and N-acetylcysteine impairs this interaction. Laboratory experiments showed that heparin and N-acetylcysteine interfere with SARS-CoV-2 infection *in vitro*. Both drugs are currently approved for use by injection and N-acetylcysteine is also approved in an inhalation formulation. The objective of the HOPE trial is to demonstrate that inhalation of heparin and N-acetylcysteine improves the pulmonary function and allows for elimination of mechanical ventilation [64].

Antifibrinolytics represent another attempt to treat haemostatic disorders in CIC. Authors have reported using tissue plasminogen activator (Alteplase) for treatment of three patients with COVID-19-associated acute respiratory failure [65]. Initially, all patients had severe acute respiratory failure (oxygenation index ($\text{PaO}_2/\text{FiO}_2$) 72, 73, and 82, respectively) and were placed on mechanical ventilation. For all these patients Alteplase was indicated because of their significantly high plasma levels of D-dimers ($> 50,000$ ng/mL, 20,293 ng/mL, and $> 33,328$ ng/mL, respectively). The drug was administered at a dose of 25 mg over 2 hours, followed by a 25 mg infusion over the subsequent 22 hours. Following thrombolysis, the oxygenation index in all patients increased up to 150, 135, and 125, respectively, but despite this treatment all of them died later.

Another treatment opportunity is eculizumab, a drug used to treat HUS, which is also associated with thrombotic events, resulting in multiorgan dysfunction. A group of authors reported using eculizumab in four patients with confirmed SARS-CoV-2 pneumonia. Eculizumab was administered once a week at a dose of 900 mg. Patients were also treated with enoxaparin 4,000 IU/day via subcutaneous injection, lopinavir 800 mg/day + ritonavir 200 mg/day, hydroxychloroquine 400 mg/day, ceftriaxone 2 g/day IV, and vitamin C 6 g/day for 4 days, and were on non-invasive continuous positive airway pressure. All patients survived and showed improvement in the lung disease. Normally, all candidates for eculizumab therapy should receive a meningococcal vaccine [66]. Because it was impossible for patients with rapidly developing COVID-19 infection, all of them received prophylaxis with ceftriaxone. It should be pointed out that in these cases eculizumab was used not to treat COVID-19-associated HUS, but to inhibit the complement system in infectious patients, i.e. as an anti-inflammatory agent. Some trials of eculizumab in patients with COVID-19 have even been announced on *ClinicalTrials.gov*. One is an open-label, multicenter trial that is being prepared by Alexion. It will include patients with a confirmed diagnosis of SARS-CoV-2 infection and severe pneumonia. They will receive up to seven (at least five) doses of eculizumab.

The total duration of the programme will be 4.5 months. Another trial announced on *ClinicalTrials.gov* is being conducted by Hudson Medical. In this trial all patients with SARS-CoV-2 infection will receive eculizumab at a standard dose of 900 mg IV over 30 minutes every 7 days and ceftriaxone IV as an alternative prophylactic antibiotic covering infection caused by *Neisseria meningitis*. Complement blood levels will be measured every 72 hours. The duration of therapy will be determined by the investigator. Patients will be followed up at days 7, 14, and 28 after discharge from hospital. The recorded assessments include mortality, time in the ICU, and time on a ventilator.

Finally, a third trial will evaluate the use of ravulizumab (Ultomiris) for the treatment of COVID-19. Ravulizumab is a new drug developed by Alexion for the treatment of HUS. It is a humanized monoclonal antibody that binds specifically and with high affinity to the complement protein C5, thereby inhibiting its activity. In fact, ravulizumab is an improved version of eculizumab with a higher specificity and a longer duration of action. The company plans to conduct a randomized, controlled trial of 270 patients with COVID-19. The following parameters will be assessed: survival at day 29, number of days free of mechanical ventilation at day 29, change from baseline in $\text{SpO}_2/\text{FiO}_2$ at day 29, duration of intensive care unit stay at day 29, change from baseline in SOFA score at day 29, survival at day 60 and day 90, and duration of hospitalisation.

Conclusion

In summary, treatment of CIC is an integral and necessary part of a combination treatment strategy for COVID-19. The effectiveness of CIC treatment influences the severity of COVID-19 infection and its prognosis.

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