Review

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COVID-19: progression of disease and intravascular coagulation – present status and future perspectives

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Abstract: The timely and accurate diagnosis of infection with severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), remains the cornerstone of efforts to provide appropriated treatment for patients, to limit further spread of the virus and ultimately to eliminate the virus from the human society. We focus this article on (a) developments for improvement of diagnosis of specific SARS-CoV-2 virus, (b) laboratory changes in the immunologic and coagulation system, (c) therapeutic options for anticoagulant treatment of seriously affected patients and (d) on the perspectives through improvement of diagnostic and therapeutic medical procedures.

Keywords: antibodies; anticoagulation; autopsy; coagulation parameters; COVID-19; D-dimer; disseminated intravascular coagulation; heparin; low-molecular-weight heparin; pulmonary embolism; SARS-CoV-2; thrombosis.

Introduction

An outbreak of coronavirus disease in late December 2019 (coronavirus disease 2019 [COVID-19]) created a pandemic of severe interstitial pneumonia, which developed within a short time frame of 3 months and involved 187 countries worldwide. COVID-19 pneumonia develops from the severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2). Previous strains of this betacoronavirus have been identified in 2003 and 2012 as causing SARS and also the Middle East respiratory syndrome (MERS). More recently, it has been shown that the SARS-CoV-2 virus migrates from nasopharygeal mucosa cells into the alveolar endothelium of the lungs being taken up via angiotensin-converting-enzyme 2 (ACE-2) receptors to be released into the blood stream. Organs with ACE-2 receptors take up the virus and cause local infections in the endothelium of the vascular system [1, 2], myocardium [3], kidney [4] and brain by passing the blood-brain barrier [5], which ultimately cause multiple fatal organ failure [6]. Autopsy of deceased patients indicates these developments of septicaemia and is likely to increase the knowledge of suspected COVID-19-related deaths [7].

Like other severe infections, COVID-19 pneumonia may induce sepsis-induced coagulopathy (SIC) and (if not controlled despite adequate medical therapy) progress to disseminated intravascular coagulation (DIC) [8]. DIC is one of the severe complications identified in patients with pneumonia, septicaemia, malignancy and other severe diseases [9]. The clinical diagnosis of DIC is made on rapid progression of serious deterioration of organ functions resulting in a boost of intravascular thrombin generation and microthrombi with secondary parenchymal bleeding through endothelial leakage [10]. Venous thromboembolism (VTE) may develop in COVID-19 patients via immunologic and toxic activation of intravascular and platelet-released thrombin [7]. The occurrence of fatal pulmonary embolism (PE) may be more frequent than that described so far and is the subject of ongoing examinations in pathology [7].

Onset of disease

The clinical manifestations of COVID-19 infection include fever, myalgia, cough and dyspnoea, and less frequently headache, diarrhoea, nausea, vomiting [11] and dysgeusia as reported in Zika virus infection [12]. Viruses spread through the bloodstream and mainly lodge in the lungs,

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gastrointestinal tract and heart, presumably concentrated in the tissues expressing ACE-2, a receptor of SARS-CoV-2. The median time from onset of symptoms to first hospital admission was calculated at 7.0 days (4.0–8.0), for shortness of breath at 8.0 days (5.0–13.0), for interstitial pneumonia at 9.0 days (8.0–14.0) and transfer to an intensive care unit (ICU) with mechanical ventilation at 10.5 days (7.0–14.0) mainly due to acute respiratory distress syndrome (ARDS). Forty-one percent of patients had comorbid chronic diseases (cardiovascular, respiratory, cancer, liver and kidney) [13]. The median time from illness onset to discharge was 22.0 days (IQR 18.0–25.0) with no difference between survivors and non-survivors [14].

Viral load and dynamics

Viral load measurements from tissue samples are indicative of active virus replication and are routinely used to monitor severe viral respiratory tract infections, including clinical progression, response to treatment, cure and relapse. Duration of viral load dynamics of posterior oropharyngeal saliva was longer in patients with severe disease (median 21 days, range 14–30 days) compared to patients with mild disease (14 days, 10–21 days) [15]. The median RNA viral load presentation of patients with clinical symptoms was 5.2 $\log_{10}$ copies per mL (range 4.1–7.0, limit of detection: 1 $\log_{10}$ copies per mL). Older age was correlated with higher viral load but not for survivors vs. non-survivors of COVID-19 [16]. Children are also carriers of the SARS-CoV-2 virus [17], but age between 1 and >45 years was not related to viral load [18].

Diagnosis of SARS-CoV-2 infection

The determination of SARS-CoV-2 by a PCR mRNA is the standard of care for objective documentation of the disease. The positive results of PCR mRNA testing decreased over time period from 67% within 7 days after onset of symptoms to 46% during day 15–39. Limitations of the performance of the PCR testing are manifold. Preanalytical errors [19], differences in primers of mRNA and isolated strains of SARS-CoV-2 and methods used for the genetic testing pose difficulties of comparability of tests [20–22].

Determination of antibodies IgG and IgM in addition to the PCR mRNA of COVD-CoV-19 was reported to improve the sensitivity and specificity of detection over 28 days after onset of symptoms. The median seroconversion time of IgM and then IgG antibodies against SARS-CoV-2 was 12–14 days, respectively. The generation of antibodies was below 40% within the first week and increased to 94% (IgM) and 80% (IgG) over 15 days [23]. Another study reported seroconversion for SARS-CoV-2 IgG and IgM antibodies in 100% of 285 patients with COVID-19 within 19 days after onset of clinical symptoms. IgG and IgM titres became positive simultaneously or sequentially and reached a plateau within 6 days after seroconversion. The authors concluded that serological testing may be helpful for the diagnosis of suspected patients with negative RT-PCR results and for the identification of asymptomatic infections [24].

Point-of-care methods are in development using the lateral flow immunoassay technique that detects IgM and IgG antibodies simultaneously using blood from a fingerprick and presenting results within 15 min. The sensitivity and specificity were 89% and 91%, respectively [25]. However, results of other rapid IgG and IgM tests could not reproduce these findings, and the test was not recommended for clinical use yet [26]. In addition, testing for antibodies only identifies a prior infection, and is inappropriate for early detection [27].

General laboratory findings

Laboratory parameters have been reported as elevated in severely diseased patients at hospital admission and/or increase with deterioration of the disease: alanine aminotransferase, lactate dehydrogenase, high-sensitive C-reactive protein, and levels of levels of IL-2R, IL-6, IL-10 and TNF-α. T lymphocytes, CD4 + T and CD8 + T cells are decreased and expressions of IFN-γ tended to be lower in non-survivors compared to survivors of the disease [28]. High plasma levels of proinflammatory cytokines (IL-2, IL-7, granulocyte colony-stimulating factor, IP10, MCP1, MIP1A, TNF-α and procalcitonin) have been observed in COVID-19 patients admitted to ICUs, suggesting that a cytokine storm effect may be developing in individuals with severe disease [11]. Elevation of neutrophils, SAA, PCT, CRP, cTnI, D-dimer, LDH and lactate levels, and the decline of lymphocyte counts, can be used as indicators of disease progression [29] (Table 1).

Diagnosis of disseminated intravascular coagulation

DIC is one of the severe complications identified in patients with pneumonia and other infections [9].

Table 1: Summary of potentially useful laboratory tests in COVID-19.[a]

Initial test set (expected outcome)	– C-reactive protein (CRP; elevated) – Lactate dehydrogenase (LDH; elevated) – Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (elevated) – D-dimer (elevated) – Fibrinogen (elevated) – PT (slight elevation) – APTT (shortened in acute phase, potentially elevated later) – Albumin (decreased) – Full/complete blood count (platelets and lymphocytes decreased)
Tests potentially useful for monitoring patient status	– CRP (monitoring of infection/inflammatory response) – LDH (identification of lung injury and/or multiple organ failure) – ALT/AST/Bilirubin (identification of liver injury) – Albumin (identification of liver failure) – Cardiac troponins (identification of cardiac injury) – Creatinine and blood urea nitrogen (identification of kidney injury and/or failure) – Procalcitonin (identification of bacterial co-infections) – Full/complete blood count (platelets, lymphocytes, neutrophils) – D-dimer, fibrinogen, PT(/APTT) (identification of ongoing [consumption or thrombotic] coagulopathy including DIC) – Electrolytes and glucose (identification of metabolic derangement) – Lactate dehydrogenase (identification of lung injury and/or multiple organ failure) – Creatine kinase (identification of muscle injury) – Lipase (identification of pancreatic injury) – Brain natriuretic peptide[b] (identification of cardiac failure) – Ferritin (monitoring of infection/inflammatory response) – Presepsin[c] (monitoring of severity of viral infection)

[a]'Gating rule': unless clinically justified, testing should not generally be reordered within 24 h of an existing test. [b]For selected patients with signs of multiple organ failure or systemic inflammatory response syndrome. Discuss with expert (laboratory) clinician/senior or clinical scientist. [c]For patients under intensive care. Modified from Favaloro and Lippi [30].

Not surprisingly, the occurrence of DIC has also been described in COVID-19-driven pneumonia. However, diagnosis of DIC is suspected by deterioration of laboratory parameters documented by repeated determinations.

The most frequently determined parameters in COVID-19 patients have been the following: prothrombin time (PT) and activated partial thromboplastin time (APTT) both increase (suggestive of coagulation activation) and decrease (consistent with consumptive coagulopathy), and fibrinogen increases (suggestive of acute-phase changes) and decreases (consumptive coagulopathy). Later stages of the disease are also characterised by increase in thrombin-antithrombin complex, fibrin-degradation products and D-dimers, with the degree of changes related with a risk of fatal outcome [31]. Platelet counts increase in the acute phase of COVID-19 disease [32] but may decrease in late stages of DIC (Table 1). About 71% of non-survivors and 0.6% of survivors showed evidence of overt DIC identified with a median time of 4 days after onset of interstitial pneumonia [33].

Of great interest is the determination of D-dimer levels. A pooled analysis including four studies showed that D-dimer values are three-fold higher in patients with severe COVID-19 than in those with milder forms. When D-dimer levels increased to levels higher than 3 μg/mL, the mortality rate increased three-fold [34]. D-dimer reached maximum levels at a median time of 4 days after onset of interstitial pneumonia in 71% of non-survivors [35]. A multivariable logistic regression model identified older age, higher SOFA score [36] and D-dimer greater than 1 μg/mL at admission to be associated with increased probability of fatal outcome [35]. Patients with COVID-19 pneumonia and PE documented by computerized tomography (CT) angiography had higher D-dimer levels compared to those without PE (median, 6.11 vs. 1.92 μg/mL). D-dimer had a sensitivity and specificity of 100% and 67%, for the presence of PE on CT angiography at a cut-off of 2.66 μg/mL, respectively [37].

Elevations of D-dimer have also been reported in severe courses of other viral infections, including human immunodeficiency (HIV) [38], Ebola [39], Zika and Chikungunya viruses [40].

Therapeutic options for treatment of DIC in COVID-19 disease

Heparins

The International Society of Thrombosis and Haemostasis reported guidelines for treatment therapeutic doses of unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) if thrombosis predominates and also in critically ill, non-bleeding patients with DIC, despite lacking direct evidence of the beneficial effects [41, 42]. The number of reports currently increases on the potential benefit of heparins in COVID-19 patients. Tang et al. reported 99 patients with COVID-19 with 94 and 5, respectively, receiving LMWH (40–60 mg enoxaparin/day s.c.) or UFH (10,000–15,000 IU/day continuously i.v.) for 7 days or longer. D-dimer, PT and age were positively, and platelet count was negatively correlated with 28-day mortality. When D-dimer exceeded 3.0 µg/mL (six-fold of upper limit of normal), mortality was 20% lower with treatment using LMWH or UFH [35]. Despite systematic thrombosis prophylaxis with low or medium dose of LMWH, 27% of COVID-19 patients admitted to the ICU developed PE as confirmed by pulmonary angio-CT. The authors conclude to recommend higher doses of LMWH for patients with COVID-19 infections admitted to the ICU [43].

Middeldorp et al. reported on the incidences of objectively documented PE and DVT in hospitalised COVID-19 patients, all of them receiving LMWH. PE occurred in 12% and 1.6% of patients treated in the ICU (n = 74) and non-ICU ward (n = 124), respectively. DVT was documented in another 27% and 1.6% of patients, respectively. Mortality was 3.3 times higher in patients with PE or DVT. Dose of LMWH was doubled in all patients on ICU after termination of reporting results. Further strategies for detecting, treating and assessing risk of VTE post-discharge in COVID-19 are warranted [44].

Despite ongoing observational and clinical studies, many centres have increased the dose of anticoagulation with LMWH to 'intermediate intensity' doses such as 0.5 mg/kg twice a day of enoxaparin. A laboratory- and clinical-based thrombosis and bleeding risk-adapted strategy was reported to the decision-making of LMWH dosage [8]. A consensus document found that 31.6% of participants supported intermediate intensity dose, 5.2% therapeutic dose, while the rest supported using standard VTE prophylaxis dose for hospitalised patients with moderate to severe COVID-19 and lack of DIC [10].

National health care professionals, scientific organisations and expert groups univocally recommended the administration of LMWH in COVID-19 patients to treat DIC and VTE and to reduce mortality [45–51].

Other anticoagulant strategies

In COVID-19, the immune system is compromised by a reduction in T- and B-cell lymphocytes and an increase in inflammatory cytokines and D-dimer. High doses of intravenous immunoglobulin IgG are one of the therapeutic options in acute thrombocytopenic disorders such as immune thrombocytopenic purpura or heparin-induced thrombocytopenia. A combination of 0.5 g/kg bodyweight IgG in combination with LMWH were given for 5 days effectively in patients with a continuous decrease in B- and T-cell lymphocytes and in D-dimer [52].

Activation of the coagulation system results in local fibrin formation together with a suppression of the fibrinolysis system [53, 54]. Administration of tissue plasminogen activator (tPA) has been reported to be effectively and safely used in three patients with COVID-19 complicated by ARDS [55]. Indeed, SARS-CoV-2 is likely to disrupt several fibrinolysis pathways, and this can be hypothesised to contribute to lung pathology and thus to adverse symptomology of breathing [56]. The bleeding risk of patients needs to be taken into consideration for treatment with tPA.

Thrombomodulin acts a receptor for thrombin activating the protein C and protein S pathway and thereby inhibiting blood coagulation through factors V and VIII. A combined analysis of three trials using intravenous recombinant human thrombomodulin reduced the 28-day mortality of COVID-19 patients with DIC by 20% but did not reach clinical significance [57].

Future perspectives

We continue to learn more about COVID-19 every day. And yet there remain many unknowns at the time of writing. The best therapeutic intervention is still under investigation. Certainly, heparin, either UFH or LMWH, is likely to be a life-saver in sufferers of severe disease. However, optimal doses are unknown, and clinicians must finely balance risk of thrombosis vs. bleeding in these patients. Of interest are the antiviral effects of heparins with low or lacking anticoagulant activity [58, 59]. Urgent studies should also be planned to define whether adjunctive antithrombotic therapies (e.g. other anticoagulants, antithrombin or thrombomodulin) may be helpful in patients with severe COVID-19. In some patients, IgG

support may also be useful. The early use of convalescent plasma therapy is also being investigated [60].

The pathophysiology of COVID-19 is complex. Most affected patients suffer only mild disease (Figure 1). In those who suffer severe disease, a multitude of events may be occurring. There appears to be an acute phase characterised by activation of coagulation, followed by or co-incident to consumptive events – thus, some patients may present with shortened APTT and elevated fibrinogen, whereas others will present with elevated APTT and reduced fibrinogen – suggesting different patterns based on what happens to be dominant in any given patient.

What is happening to platelets is also fascinating in these patients; again, both elevations and reductions in platelet count seem to be present in different patients. There is likely initial platelet activation and subsequent clearance of platelets from circulation. Platelets are also known to bind to various viruses and this is a proposed general cause of platelet depletion in infection [61]. The situation with SARS-CoV-2 and platelets is yet to be resolved, but likely to be similar.

Finally, there can be some recommendations for tests to be performed on severely affected patients that may have some prognostic value. A recent publication has provided such a list [30], whilst recognising that the situation remains dynamic and subject to change.

Signal for the urgent need of diagnostic, therapeutic, medical, social and other aspects is given by the large number of over 450 registered clinical trials worldwide (ClinicalTrials.gov). The ultimate information on the

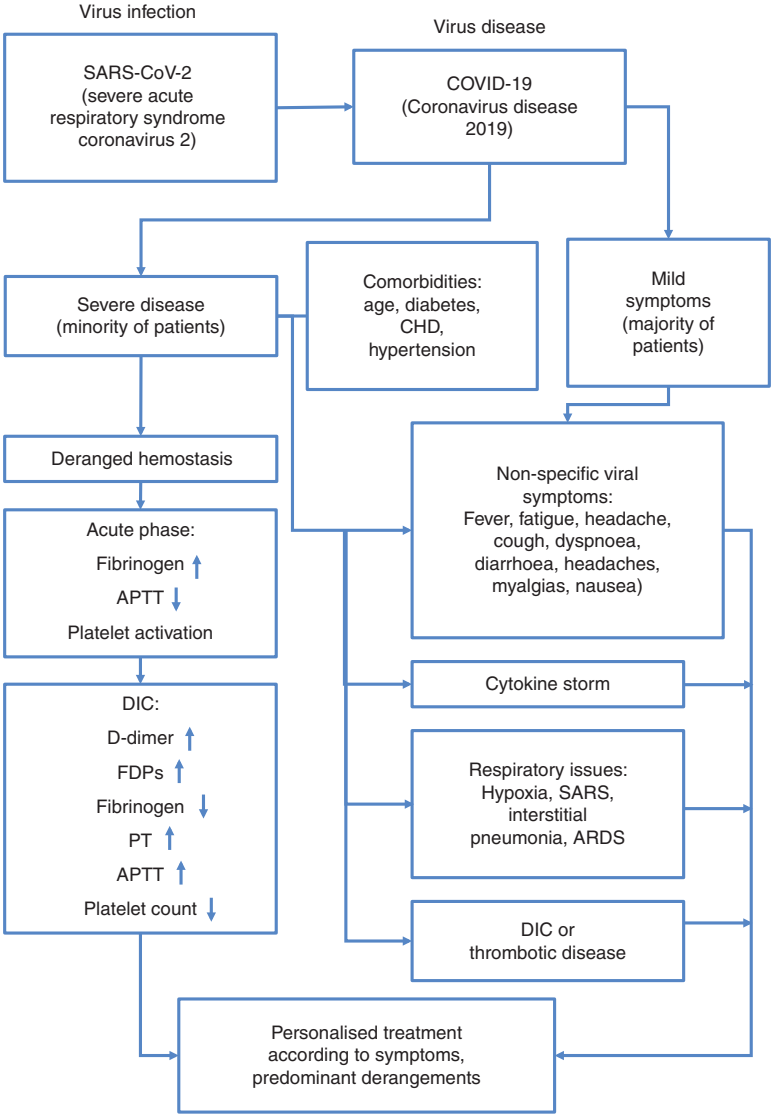

Figure 1: Summary of concepts detailed in the current review.

multiple causes of death of COVID-19 patients by SARS-CoV-2 virus will be obtained by autopsy of patients with not clearly defined cause of death [62–64].

Conclusions

Based on the deterioration of coagulation and also the immunologic system, patients with COVID-19 deteriorate and suffer from severe disease. DIC is one of the main complications that evolves rapidly, and that can be diagnosed within a short time frame of a few days. The multifactorial pathophysiology of DIC results in multiple therapeutic options currently available for treatment. However, pathophysiology of COVID-19 is complex (Figure 1), and not all patients may have the same 'disease'. Thus, although adequate use of at least one of the likely therapeutic options of UFH or LMWH and sequential determination of D-dimer should be followed, specific treatment for each patient may require specific identification of symptomology causality to enable safe prophylaxis and treatment of all individuals with COVID-19, and along the concept of personalised medicine. Increasing rates of autopsies of patients with not objectively confirmed causes of death will improve the understanding of COVID-19 disease to improve the healthcare system and of the welfare of the population.

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