**COVID-19 and Immunothrombosis: emerging understanding and**

**clinical management**

**Short title:** COVID-19 and Immunothrombosis

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***Abstract***

The COVID-19 pandemic has been the most significant health crisis in recent global history. Early studies from Wuhan highlighted COVID-19-associated coagulopathy and a significant association with mortality was soon recognised. As research continues across the world, more evidence is emerging of the cross-talk between the innate immune system, coagulation activation and inflammation. Immunothrombosis has been demonstrated to play a key role in the pathophysiology of severe COVID-19, with extracellular histones and neutrophil extracellular traps detected in the plasma and cardiopulmonary tissues of critically ill patients. Targeting the components of immunothrombosis is becoming an important factor in the treatment of patients with COVID-19 infection. Recent studies report outcomes of **intermediate and therapeutic** anticoagulation in hospitalised patients with varying severities of COVID-19 disease, including optimal dosing and associated bleeding risks. Immunomodulatory therapies, including corticosteroids and IL-6 receptor antagonists, have been demonstrated to significantly reduce mortality in critically ill COVID-19 patients. As the pandemic continues, more studies are required to understand the driving factors and upstream mechanisms for coagulopathy and immunothrombosis in COVID-19, and thus potentially develop more targeted therapies for SARS-CoV-2 infection, both in the acute phase and in those who develop longer term symptom burden.

**Background**

Since it first emerged in December 2019 in Wuhan, Hubei province, the highly infectious severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has spread across the globe causing the coronavirus disease (COVID-19) pandemic and claiming more than 2 million lives by February 2021.1 Although around 80% of people will experience a mild to moderate illness, a proportion of those infected develop a profound hyperinflammatory response with striking pro-thrombotic tendencies; 15% experience severe illness (dyspnoea, hypoxia or >50% lung involvement on imaging) and 5% critical illness (respiratory failure, shock or multi-organ failure)2 and overall COVID-19 carries a mortality of approximately 2.2% globally.1 **Immunothrombosis is a component of the innate immune system, triggered by pathogens or cell damage, whereby activation of coagulation occurs secondary to inflammation and microthrombi are formed within small vessels.**3Interactionsbetween the innate immune system, coagulation activation and endothelial dysfunction are evident in COVID-19 and as understanding of SARS-CoV-2 increases, it is becoming clear that immunothrombosis plays a pivotal role in the pathogenesis of severe COVID-19 disease.4 Consequently, the multi-faceted role of heparin, immunomodulatory therapies and potential novel therapeutic targets have been considered in the management of COVID-19. This review summarises the evidence to date and highlights emerging considerations.

***Interaction between SARS-CoV-2 and the endothelium***

SARS-CoV-2 is an RNA coronavirus predominantly transmitted in humans via the respiratory tract. SARS-CoV-2 enters and infects host cells via interaction between the spike protein binding domain and the ACE-2 receptor on the host cell membrane.5 Such interactions cause internalisation of ACE-2 and can disturb the balance between ACE and ACE-2. ACE/ACE-2 imbalance increases the action of angiotensin II6 and the potential for pulmonary vascular contraction, inflammation and thrombosis. It also increases plasminogen activator inhibitor-1 levels, which inhibits tissue plasminogen activator (tPA) to reduce fibrinolysis.7, 8 A hypofibrinolytic state can enhance fibrin deposition in alveolar tissues and the microvasculature, and promote development of acute respiratory distress syndrome (ARDS).9 The lungs are the most susceptible organ with high expression of ACE-2 by pneumocytes in the epithelium of alveolar tissue. However, ACE-2 receptors are also expressed on endothelial cells of extrapulmonary organs including the gut, heart and kidney as well as the vascular endothelium.10-13 Autopsy studies of lungs of COVID-19 patients demonstrate severe endothelial injury as well as intracellular virus and disrupted cell membranes.14 Interestingly, the expression of ACE-2 from the nasal epithelium in particular appears to be age dependent,15 and the reduced ACE-2 expression in the paediatric cohort may account for the lower prevalence of COVID-19 in children.2 **SARS-CoV-2 infection in children is generally very mild, however, a small proportion of the paediatric cases develop a rare severe hyperinflammatory syndrome with multi-organ failure termed ‘multisystem inflammatory syndrome in children’ (MIS-C) around 4-6 weeks after infection.16, 17 In rare cases, even some adults have been reported to develop signs and symptoms similar to MIS-C occurring weeks after COVID-19 infection.18** Generally, the rates of hospitalisation and deaths from COVID-19 are higher in those aged 65 years or older as well as patients with pre-existing cardiometabolic diseases,19 where underlying endothelial dysfunction is a unifying feature.20

***Thrombotic complications in COVID-19***

A high incidence of thrombotic complications, **including venous, arterial and microvascular thrombosis**, is recognised in COVID-19. **Thrombosis is seen** particularly in those patients who are critically ill21 **and** these complications appear to be occurring despite conventional low dose venous thromboembolism (VTE) prophylaxis.22 The most critically ill patients with SARS-CoV-2 infection develop ARDS, with respiratory failure being the leading cause of mortality.23 Severely ill COVID-19 patients have a median time from onset of dyspnoea to intubation of 10.0 days (IQR 5.0 – 12.5 days)24 and median time to intensive care (ICU) admission of 9.5 days (IQR 7.0 – 12.5 days),25 thus highlighting the potential for patients to rapidly deteriorate.

**A global systematic review and meta-analysis of VTE rates in patients with COVID-19 demonstrated the presence of thrombosis in 22.7% of critically ill patients admitted to ICU and 7.9% of hospitalised (non-ICU) COVID-19 patients.**21 An observational study from the Netherlands reports a high incidence of confirmed VTE in 198 patients with COVID-19 admitted to hospital (particularly within the critical care setting). All patients received standard dose thromboprophylaxis as part of their care. Overall, 20% of patients were diagnosed with VTE (of which 13% were symptomatic) and VTE was observed in 35 of 74 ICU patients (47%). Of the ward based patients, 4 of 123 (3%) were diagnosed with VTE despite receiving thromboprophylaxis.26

Although patients admitted to critical care already have a number of acquired risk factors for venous thromboembolism including mechanical ventilation, prolonged periods of immobility and venous catheter insertion,27, 28 the addition of COVID-19 infection exceeds the thrombotic risk which is seen in other respiratory viral illnesses.26 Helms et al. compared the incidence of VTE in patients with COVID-19 related ARDS and a historical cohort of non-COVID ARDS. COVID-19 ARDS patients (n = 77) developed significantly more thrombotic complications than non-COVID-19 ARDS, primarily consisting of pulmonary embolism (11.7 vs. 2.1%, p < 0.008).29 Dual-energy CT imaging has been used to further examine lung perfusion and investigate for pulmonary embolism and infarction in COVID-19.30 Some case studies suggest that scan appearances are more in keeping with pulmonary thrombosis in situ rather than pulmonary embolism.31 Significant difficulties with extracorporeal circuits on machines used in intensive care has also been observed and Helms et al. reported that 28 of 29 (96.6%) patients on continuous renal replacement therapy experienced this complication.29

Thrombosis in more unusual sites has been reported in small case series of patients with COVID-19 infection, particularly extensive cerebral venous sinus thrombosis, as a neurological complication.32, 33 Although the majority of thrombotic events reported in COVID-19 are venous, arterial events are also recognised, including large vessel stroke in younger patients.34 Acute coronary syndrome (ACS) can also be a complication or a presentation of COVID-1935, 36 and contributes to the mortality. A large study of 3334 hospitalised COVID-19 patients demonstrated myocardial infarction (MI) was the commonest thrombotic event in the cohort, occurring in 8.9% of patients.37 Additionally, a small case series of COVID-19 patients undergoing primary coronary interventions for ST elevation MI showed neutrophil extracellular traps (NETs) formation in 68% of thrombi.38

In addition to larger vessel thromboses, examination of the lung and cutaneous tissue autopsy samples from patients who died from severe COVID-19 infection and respiratory failure demonstrates the presence of microvascular injury with thrombosis.39, 40 Diffuse inflammatory alveolar change with macrophage infiltration has been reported in early pathological studies of patients with COVID-19 pneumonia, alongside extensive vascular congestion.41 Fox et al. discovered widespread thrombotic microangiopathy within the pulmonary vasculature. Fibrin thrombi were found within distended small vessels/capillaries alongside extensive extracellular fibrin deposition.42 These extensive inflammatory changes seen within the lungs as well as the marked microvascular thrombosis seen within vessels have been termed “diffuse pulmonary intravascular coagulopathy” to emphasise the more localised nature of COVID-19 coagulopathy.8, 43 **There is variation in the reported microthrombosis rates between 45-100% of COVID-19 patients.44-46 Carsana et al report platelet-fibrin thrombi in the lung tissue of 87% of patients who died from COVID-19;45** **Wichmann et al demonstrated pulmonary microthrombi in autopsy lung specimens from COVID-19 patients (N=12/12),** they also demonstrated a high incidence of thromboembolic events, including deep vein thrombosis in seven patients (58%) in whom venous thromboembolism was not suspected prior to death, as well as four cases where pulmonary embolism was the direct cause of death.46 This highlights thrombosis as a significant contributor to death in COVID-19. **Nicolai et al. compared histological specimens from lungs of deceased patients with COVID-19 to those with influenza pneumonia (H1N1 or seasonal). A significant difference in neutrophil extracellular trap formation and immunothrombotic occlusion of microvessels was demonstrated in COVID‐19 compared to influenza (40.8% ± 5.4 of vessels affected versus 9.4% ± 4.0 respectively).47**

In patients with COVID-19 infection, microvascular injury and inflammation can present as a multitude of skin rashes,48, 49 particularly those of a vascular nature such as purpuric lesions.50 Zhang et al. reported seven cases of patients with severe COVID-19 infection on intensive care who developed ischaemia of the digits.51 Histopathological examination of skin biopsies from a paediatric cohort with chilblains (“COVID toes”) showed the presence of coronavirus particles within the endothelium with associated vascular damage.52 Magro et. al., examined skin and lung tissue from five patients with severe COVID-19 infection and found pauci-inflammatory septal capillary injury with significant septal capillary mural/luminal fibrin deposition as well as permeation of the inter-alveolar septa by neutrophils as a dominant feature in the lungs, with significant deposits of complement membrane attack complexes (MAC) in the microvasculature. Similar patterns were found in purpuric skin rashes to suggest that the immune response, including complement activation, plays an important role in thrombus formation in COVID-19.53 **Moreover,** **Carvelli et al. demonstrated the significance of complement activation showing levels of soluble C5a increase in proportion with the severity of illness in patients with COVID-19.**

***Laboratory findings of systemic inflammation, immune cell death and coagulation activation***

**COVID-19-associated coagulopathy was recognised early in the pandemic and understanding has been refined such that it is recognised as a separate clinical entity to disseminated intravascular coagulation (DIC). Elevated D-dimer is a common observation**,10 with a 2 - 5 fold elevation in severe COVID-1954, 55 indicating coagulation activation. **Other key features of COVID-19 were recognised in early data from Wuhan including elevation in acute phase proteins and inflammatory markers, particularly in those who were critically ill and many of which are predictors for mortality.**

Systemic coagulopathy is a key feature of morbidity and mortality in COVID-19 and correlates with the severity of disease.56, 57 **The biochemical features of COVID-19-associated coagulopathy include elevated D-dimer, elevated fibrinogen, mildly prolonged/normal prothrombin time and mildly reduced/normal platelet count.** Tang et al. studied 183 patients with novel coronavirus and compared the coagulation parameters between survivors and non-survivors; on admission, non-survivors were found to have a significantly higher D-dimer, fibrin degradation products (FDP) levels and a mildly prolonged prothrombin time (PT) compared to survivors.56 These observations have been echoed in findings from Guan et al. who demonstrated D-dimer was elevated in 46% of patients with COVID-19 admitted to hospital, and a higher proportion of those with a more severe disease phenotype (e.g. those requiring ventilation and non-survivors) had a raised D-dimer (59.6% versus 43.2%).58

Although fibrinogen is generally elevated on admission (likely due to the acute phase response), later in the disease course of COVID-19 non-survivors show a significantly lower fibrinogen and mildly reduced antithrombin levels compared to survivors.56 Mild to moderate thrombocytopenia is also recognised.24, 58 The pattern of parameters in COVID-19 associated coagulopathy **certainly** has notable differences to the picture of DIC, **including** a milder thrombocytopenia and less pronounced prolongation of the PT.

Elevated plasminogen has been described in severe COVID-19 infection,59 which may be in response to fibrin deposition in the lungs of infected patients and subsequent dysregulation of the coagulation and fibrinolytic systems.9 Hypercoagulable thromboelastometry profiles were observed in a small cohort of COVID-19 patients, reflected by shorter clot formation time in INTEM and EXTEM and higher maximum clot firmness (MCF) in INTEM, EXTEM and FIBTEM, thus indicating a severe hypercoagulability rather than consumptive coagulopathy.60 Significant elevation of Factor V, VIII, and von Willebrand factor (VWF) have also been demonstrated61, 62 in patients with severe COVID-19 infection. High plasma levels of VWF antigen, as well as VWF propeptide, demonstrated in severe COVID-19 are suggestive of endothelial stimulation and damage, with release of VWF from Weibel-Palade bodies.63, 64

Acquired coagulation changes due to anti-phospholipid antibodies have been described. Bowles et al. reported on a cohort of 34 COVID-19 patients with prolonged aPTT; 91% demonstrated the presence of lupus anticoagulant by dilute Russell’s viper-venom time [DRVVT] and lupus anticoagulant-sensitive aPTT.65 Anticardiolipin and B2GP1 **IgG or IgM antibodies have been shown to be present in 10% of hospitalised patients in one study of 50 patients66** but it remains unclear whether these antibodies are persistent or transient in nature as is common with other infections.67

As highlighted in the early studies from China, LDH - an indicator of cell death - was found to be significantly more elevated in patients admitted to ICU vs. those who did not require critical care (400 U/L vs 281 U/L, P = 0.0044).10 Likewise, LDH is significantly more elevated in severe COVID-19 infection vs. non-severe infection (424 U/L vs 204 U/L, P <0.001).68 Lymphopenia is commonly observed in tandem to suggest immune cell death and its severity correlates to more severe disease requiring intensive care/ventilatory support and in non-survivors.10, 55, 58, 68-70 Other commonly observed changes include those marking the acute phase response and inflammation such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) with elevated levels in non-survivors compared to survivors of COVID-19.69-71

Several key predictors for mortality in COVID-19 have been reported, including lymphopenia, high LDH, high CRP and high D-dimer, as well as organ injury markers, such as elevated cardiac troponin. Huang et al. first reported that D-dimer was 5-fold higher than the normal range in severe COVID-19 in 41 patients hospitalised in Wuhan, China10 and elevated D-dimer was an independent predictor for mortality in hospitalised patients with COVID-19.56, 72 Zhou et al. proposed a D-dimer value of 1μg/ml for predicting mortality,24, 73 with other groups using a higher cut off for D-dimer for example: 1.5 μg/ml to achieve 85.0% sensitivity and 88.5% specificity, with 94.7% negative predictive value22 versus 2.0 μg/ml to predict hospital mortality (HR 51.5, 95%CI:12.9-206.7).72

***Potential drivers of immunothrombosis in COVID-19***

Thrombosis as a component of the innate immune response against pathogens is called immunothrombosis3, 74 and can be a potentially fatal complication of infection.75 Neutrophil recruitment and activation with subsequent NETosis, endothelial cell damage and activation, and platelet activation and aggregation, together with coagulation protease activation, all participate in the complex process of immunothrombosis, especially in the lungs. This forms the central pathogenic process of ARDS.76 In recent years, the roles of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), in particular extracellular histones and DNA, have been explored in the development of immunothrombosis. PAMPs and DAMPs increase tissue factor (TF) expression on monocytes and induce NETs formation to promote immunothrombosis.77, 78 Cell-free DNA (cfDNA) and extracellular histones, the key components of NETs released from cell death, enhance host inflammation and induce thrombosis by impairing fibrinolysis, increasing thrombin generation and promoting platelet activation.78, 79

Severe sepsis, as the host’s response to infection and inflammation, promotes activation of the coagulation system in addition to down-regulation of the natural anticoagulant proteins and fibrinolysis.80 Furthermore, there is endothelial dysfunction including increased expression of adhesion molecules and increased vascular permeability which in turn contributes to a prothrombotic state.80, 81 TF expression is increased following proinflammatory cytokine release and acquired protein C deficiency develops due to a combination of consumption, increased degradation and reduced synthesis.82-84 Essentially, sepsis is a syndrome of multiple organ dysfunction which occurs in response to acute bacterial or viral infection, with concurrent activation of the innate immune, inflammatory and coagulation systems; ultimately leading to disseminated microvascular thrombosis to induce multiple organ failure.85, 86

Other viral infections, such as SARS and Ebola, have shown vascular endothelial damage in both small and medium-sized pulmonary vessels. DIC, deep vein thromboses and pulmonary emboli are common and overexpression of TF in monocytes/macrophages may well play a role.87, 88

Severe COVID-19 infection leads to systemic hyperinflammation similar to macrophage activation syndrome or cytokine storms. It is characterised by increased plasma concentrations of interferon γ (IFNγ), IFNγ-inducible protein 10, tumour necrosis factor α (TNFα), interleukin (IL) 1β, IL2, IL6, IL7, IL8, IL10, IL17, monocyte chemoattractant protein 1 (MCP1), and macrophage inflammatory protein (MIP) 1α.10 In general terms, viral RNA has been shown to activate TLR-3 and 7 to enhance NF-κB pathway and the interferon regulatory factors (IRFs), which consequently increases the synthesis and release of pro-inflammatory cytokines.89 We know that elevated IL-6 stimulates coagulation by increasing thrombin generation90 and anti-IL-6 antibody tocilizumab has shown beneficial effects when it was used in COVID-19 to target both inflammation and coagulation.91-93 Elevated IL-1, IL-6, and TNFα could activate endothelial cells to promote thrombosis86, 94 and elevated TNFα and angiotensin II have been implicated in the enhancement of tissue factor overexpression in platelets and macrophages. In addition, damaged alveolar endothelial cells also expose tissue factor to promote fibrin deposition and thrombosis.95

Elevated IL-8 increases neutrophil recruitment and has been demonstrated to induce formation of NETs,96, 97 which form a scaffold to promote thrombosis.98 In intensive care patients, it has been previously demonstrated that the degree of NETs formation is significantly associated with multiple-organ injury and independently predicts the development of DIC and mortality.97 Elevated levels of myeloperoxidase as a NETs related marker99 have been shown in the sera of COVID-19 patients, and Barnes et al. showed infiltration of neutrophils in COVID-19 autopsy specimens.100 Middleton et al. subsequently demonstrated NET-containing microthrombi with neutrophil and platelet infiltration in pulmonary autopsy samples.101 Further work is needed to continue to investigate the important role NETs play in thrombosis formation during SARS-CoV-2 infection.

NETs release free histones and DNA, which can also be released following other types of cell death, including lymphocyte death from cytokine storms in COVID-19.102 Extracellular histones are small positively charged proteins, typically found within the cell nucleus, which bind to negatively charged DNA. Circulating histones are powerful drivers of coagulation by activating platelets, assembling prothrombinase to generate thrombin and damaging endothelial cells.79, 103 Our recent data (in press) demonstrates that circulating histones on admission to hospital were significantly elevated with increasing severity of COVID-19 infection (Mild, median=2.6µg/ml [IQR=0.7-7.6], Moderate, 10.5µg/ml [3.5-27.2], Critical, 20.0µg/ml [6.2-33.0], Non-survivors, 29.6µg/ml [11.2-60.0]; P<.001). Extracellular histones associated significantly with severe coagulopathy, inflammatory and organ injury markers, thus suggesting circulating histones may play a key role in coagulation activation and immunothrombosis formation in COVID-19 infection. We also demonstrate that extracellular histone levels on admission are associated with poor outcomes in hospitalised COVID-19 patients and independently predict 28-day mortality.

There is no doubt that multiple factors will contribute to immunothrombosis in COVID-19. Henry et al. proposed an immunothrombosis model of COVID-19 describing the underlying pathogenesis and the interaction between multiple systems, including immune response to the virus, hyperinflammation, complement activation, renin-angiotensin system, hypoxic environment, endothelium and platelets, but with no detailed links to connect these systems.7 Here, we propose an updated model for immunothrombosis in COVID-19 (Figure 1). SARS-CoV-2 infection disturbs ACE/ACE2 balance to induce hyperinflammation, cell damage (including direct viral infection of endothelial cells), triggering the immune response, proinflammatory cytokine storms and the acute phase response. This leads to recruitment and activation of immune cells, including neutrophils and macrophages. NETosis and lymphocyte death is induced, which can release DAMPs including extracellular histones. Extracellular histones are procoagulant103 and cytotoxic and mediate multiorgan injury.104 We propose that these factors act together to initiate and amplify the activation of coagulation, fibrin deposition and immunothrombosis.

**Figure 1. Mechanisms underlying immunothrombosis in COVID-19.** TF: tissue factors; TLR: Toll-like receptors; PAI-1: plasminogen activator inhibitor-1; cfDNA: cell free DNA; PAMPs: pathogen associated molecular patterns; DAMPs: damage-associated molecular patterns; IFN: interferon.



## *Management strategies in COVID-19*

Whilst there was a lack of treatment options available early in the COVID-19 pandemic, data from the large multicentre open label RECOVERY study (Randomised Evaluation of COVID-19 Therapy) was the first to demonstrate a significant reduction in 28-day mortality amongst hospitalised patients with severe COVID-19 infection who received dexamethasone 6mg for 10 days. Of the participants requiring mechanical ventilation, 29.0% of these died within 28 days compared with 40.7% in the control arm (P < 0.001); 21.5% of participants receiving dexamethasone who required oxygen therapy died within 28 days compared with 25.0% in the control group (P = 0.002).105 The release of these results led to the early-halt of the REMAP-CAP corticosteroid domain, which nevertheless showed consistent benefit of corticosteroids in COVID-19 patients. REMAP-CAP demonstrated in patients with severe COVID-19 disease, treatment with 7 days of fixed-dose hydrocortisone (and more prolonged shock-dependent dosing of hydrocortisone) versus no hydrocortisone, led to 93% (and 80%) probability of superiority in the odds of improvement in organ support–free days within 21 days.106 These results from the RECOVERY and REMAP-CAP studies demonstrate anti-inflammatory therapy has greater benefit in the treatment of COVID-19 than the trialled anti-viral therapies alone. This is also reflected in results from the REMAP-CAP study evaluating the IL-6 receptor antagonists, tocilizumab and sarilumab. Gordon et al. showed improved survival in the pooled IL-6 receptor antagonist groups, with a hazard ratio of 1.61 when compared to control group (95% credible interval, 1.25 to 2.08). The median number of organ support–free days with tocilizumab was 10 days (IQR −1 to 16), with sarilumab, 11 days (IQR 0 to 16) and 0 days in the control group (IQR −1 to 15).107 The RECOVERY study similarly showed benefit from a single dose of tocilizumab, which reduced in-hospital mortality (28% of the tocilizumab recipients vs. 36% of standard care recipients died) and time to hospital discharge (HR 1.41; 95% credible interval, 1.18–1.70) and increased the organ support-free days (10 days in the tocilizumab arm vs. 0 days in the usual care arm; OR 1.64; 95% credible interval, 1.25–2.14) (Table I).108 Subsequently, tocilizumab in combination with dexamethasone has been recommended in certain hospitalised patients who are exhibiting rapid respiratory decompensation due to COVID-19.

Globally, many guidelines were published on prevention of VTE and anticoagulation in COVID-19 disease, with widely varying recommendations predominantly based on expert consensus. More recently, interim results (in preprint) from multiplatform randomised control trials (including REMAP-CAP, ATTACC and ACTIV-4a) report that for patients with moderate COVID-19 (hospitalised, not on ICU organ support) therapeutic dose heparin was superior to standard care venous thromboprophylaxis for the primary outcome of organ support free days at day 21 (3 days (IQR –1, 16) versus 5 days (IQR –1, 16; adjusted odds ratio 0.87, 95% credible interval 0.70-1.08) with a non-significant absolute reduction in mortality of 2%. For patients with severe COVID-19 (on ICU organ support at baseline) however, therapeutic anticoagulation met the pre-defined criteria for futility thus suggesting therapeutic anticoagulation may not alter the disease course if it is initiated too late when immunothrombosis has already progressed. In patients with severe COVID-19, major bleeding occurred in 3.1% of patients receiving therapeutic anticoagulation versus 2.4% of patients receiving standard care thromboprophylaxis.109 **Recent results from the INSPIRATION randomised clinical trial showed that for COVID-19 patients admitted to ICU, the use of intermediate-dose prophylactic anticoagulation versus standard-dose prophylactic anticoagulation, did not result in a significant difference in venous or arterial thrombosis, treatment with extracorporeal membrane oxygenation, or mortality within 30 days. The major limitation of this study is the dosing in the intermediate group was assigned 1mg/kg once daily, rather than a lower dose twice daily.110**

In addition to anticoagulation, heparin also has anti-inflammatory effects111 and has even shown potential anti-viral effects in pre-clinical trials in COVID-19.112 Heparin also acts to prevent histone-mediated cytotoxicity and has been shown to improve survival in sepsis.113 This may have relevance to recent findings on the role of extracellular histones in severe COVID-19 (in press).

**Antiplatelet therapies have also been considered, a multivariate analysis using propensity score-matched patients by Meizlish et al. shows that administration of in-hospital aspirin was associated with significantly lower cumulative mortality in COVID-19 patients (HR=0.522 [0.336-0.812], p=0.004).114**

Pulmonary embolism, particularly multiple, small pulmonary vascular occlusions were commonly found at autopsy and significantly contributed to ARDS and mortality. These patients were usually critically unwell or had shown a sudden deterioration and thereby required intensive care. As discussed, CTPA was used for detecting pulmonary thrombosis/embolism in COVID-19 patients and standard therapy normally improved the outcomes.73 The diagnosis of venous thromboembolism can be very challenging in critically ill patients; they may be too unstable to transfer for confirmatory imaging or have symptoms which overlap with those of SARS-CoV-2 infection itself. Overall, we should maintain a high index of clinical suspicion for venous thromboembolism in COVID-19 and a low threshold for diagnostic imaging where feasible. Robust evidence of risk involving therapeutic anticoagulation is evolving in COVID-19, and there appears to be a low reported incidence of major bleeding115 or DIC with bleeding events;29, 56, 116 only a small fraction of COVID-19 patients truly developed DIC or overt bleeding, but these were strongly associated with mortality.57 Fibrin deposition in the pulmonary microvasculature is considered as a contributory cause of ARDS.117 To degrade pre-existing fibrin by enhancing fibrinolysis could be important and tPA is currently in clinical trials in severe COVID-19 patients but again may risk severe bleeding complications.9

***Conclusions: what does the future hold with COVID-19?***

COVID-19 is a new infectious disease with a variety of clinical presentations. However, the underlying molecular and pathophysiological mechanisms are not yet fully understood. Evidence of COVID-19 associated coagulopathy and its management is constantly evolving and calling for prompt action. The anticoagulation arm of REMAP-CAP has been adapted to **pursue investigation of** low versus intermediate dose of heparin in critical care as well as the optimal dose for those patients admitted with moderate COVID-19 disease whose condition later deteriorates, **further** consideration also needs to be given to the role of antiplatelets alongside heparin and the impact of antiplatelets on outcomes in COVID-19.118 **A multitude of clinical trials for COVID-19 therapies targeting inflammation and thrombosis are underway globally, the ACTIV (Accelerating COVID-19 Therapeutic Interventions and Vaccines) studies alone are currently studying immune modulators (infliximab, abatacept and Cenicriviroc), long-acting monoclonal antibody (investigational product AZD7442), antithrombotics (outpatient apixaban and aspirin, as well as unfractionated heparin and P2Y12 inhibitors) in phase 3 trials as well as phase 2 trials for monoclonal antibodies Risankizumab and Lenzilumab.119**

Importantly, which factors are involved in driving coagulopathy in COVID-19 is a fundamental question and cannot be fully explained by either the virus itself or the ACE/ACE2 imbalance. Circulating histones, released following cell death, have the capacity to cause coagulopathy in other critical illnesses and are associated with mortality in sepsis.120 Further studies to explore blocking upstream of coagulopathy, such as inhibiting immune cell death or neutralizing extracellular histones, may obtain better therapeutic effects than anticoagulants alone. As more mechanisms are investigated, our understanding of the development of immunothrombosis will continue to improve and further targeted therapies can be developed.

Whilst the acute effects of COVID-19 have been well described and many acute therapies trialled over the last 12 months, less is understood of the longer-term symptom burden affecting patients after recovery from the acute phase of viral illness. The symptoms, which are broadly termed “long COVID”, are widely varied but particularly include cardiovascular and pulmonary complications and have a significant impact on quality of life of patients.121 Vechi et al. report a case series of thrombotic events occurring 3-4 weeks following recovery and apparent clinical improvement from COVID-19.122 Given these findings and the significant burden of immunothrombosis seen in hospitalised patients, results from the ongoing phase 3 clinical trial “HEAL-COVID” studying 14 days of low dose apixaban versus atorvastatin versus standard care post discharge, will be eagerly awaited.123

In the meantime, the world’s focus remains on the COVID-19 vaccination roll-out. **More than 1 billion doses of vaccines have been administered globally as of early May** 2021.1 **There have been rare reports worldwide** of a syndrome, with similarities to the immunologically-mediated heparin-induced thrombocytopaenia, **which has been termed vaccine associated or vaccine induced thrombosis and thrombocytopenia (VATT/VITT). The incidence is estimated to be 1 in 100,000 people vaccinated124 and is affecting patients of all ages and both genders. The syndrome has been reported in 39 patients in the New England Journal of Medicine and consists of venous thrombosis (often in unusual sites including cerebral sinus venous thrombosis and splanchnic vein thrombosis) with concurrent thrombocytopenia occurring 5 to 14 days following 1st dose of ChAdOx1 nCoV-19 adenoviral vector vaccine.125-127 Patients are reported to have very high levels of D-dimer and hypofibrinogenaemia and importantly, almost all patients studied had high levels of anti-platelet factor 4 antibodies identified by enzyme-linked immunosorbent assay (ELISA) in the absence of prior exposure to heparin therapy.125 Current expert advice in the UK recommends treatment with intravenous immunoglobulin, to anticoagulated with non-heparin anticoagulants and consider plasma exchange therapy in very severe or resistant cases,128 but these management guidelines may change as further evidence emerges with time.**

The spate of data, experiments and insights has been profoundly shaped by the pandemic and our understanding of medicine has grown by leaps and bounds. It has brought the relevance of immunothrombosis in acutely unwell patients to the forefront with the promise of new and better treatments in the future. Our further understanding of the cross-talk between coagulation, inflammation and innate immune activation holds promise for improved and even individualised approaches, especially for those who have thrombosis and high haemorrhagic risk.

**Author contributions**

RJS wrote manuscript, CB wrote management section and critically reviewed the manuscript, GW wrote the drivers of immunothrombosis section and critically reviewed the manuscript, CHT provided overall supervision, wrote and critically reviewed the manuscript.

**Disclosures**

All authors have completed ICJME conflict of interest disclosures form and have no conflict of interests to declare.

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**Table I. Summary of evidence for therapies that target inflammation and coagulation in COVID-19 in the UK**

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Study group | Study population & sample size | Study summary |
| Dexamethasone | Dexamethasone in Hospitalized Patients With COVID-19— (RECOVERY Trial) | Multi-centre randomised open-label adaptive trial in hospitalised patients with suspected/confirmed COVID-19 (n=6,425). United Kingdom | * 28-day mortality 22.9% with dexamethasone arm vs. 25.7% in standard care arm (age-adjusted rate ratio 0.83; 95% CI, 0.75–0.93; P < 0.001).
* Greatest survival benefit among participants requiring invasive mechanical ventilation. Among these participants, 28-day mortality was 29.3% in dexamethasone arm vs. 41.4% in standard care arm (rate ratio 0.64; 95% CI, 0.51–0.81).
 |
| Hydrocortisone | Effect of Hydrocortisone on Mortality and Organ Support in Patients With Severe COVID-19: The REMAP-CAP COVID-19 Corticosteroid Domain Randomized Clinical Trial (CAPE COD) | Randomised, multi-national, adaptive platform trial of patients with severe COVID-19 (n=403) | * No difference between groups in organ-support free-days at D21 (median of 0 days in each group).
* Compared to no hydrocortisone, median adjusted OR for organ-support free days: OR 1.43 (95% credible interval, 0.91–2.27) with 93% Bayesian probability of superiority for the fixed-dose hydrocortisone group, OR 1.22 (95% credible interval, 0.76–1.94) with 80% Bayesian probability of superiority for the shock-based dosing hydrocortisone group
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| Tocilizumab (IL-6 antagonist) | Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): preliminary results | Randomised, controlled, open-label, platform trial | * Patients receiving tocilizumab were more likely to be discharged from hospital alive within 28 days (54% vs. 47%; rate ratio 1·22; 95% CI 1·12-1·34; p<0·0001).
* Amongst patients not mechanical ventilated at baseline, patients receiving tocilizumab were less likely to reach the composite endpoint of invasive mechanical ventilation or death (33% vs. 38%; risk ratio 0·85; 95% CI 0·78-0·93; p=0·0005).
 |
| Tocilizumab (IL-6 antagonist) | The REMAP-CAP investigators | Multifactorial adaptive platform trial (part of the REMAP-CAP trial), open-label | * Median organ support-free days 10 (interquartile range [IQR] −1, 16) for tocilizumab versus 0 (IQR −1, 15) for control.
* Hospital mortality was 28.0% (98/350) for tocilizumab versus 35.8% (142/397) for control group.
 |
| Sarilumab (IL-6 antagonist) | The REMAP-CAP investigators | Multifactorial adaptive platform trial (part of the REMAP-CAP trial), open-label | * Median organ support-free days were 11 (IQR 0, 16) for sarilumab versus 0 (IQR −1, 15) for control, respectively.
* Hospital mortality was 22.2% (10/45) for sarilumab versus 35.8% (142/397) for control group.
* All secondary outcomes and analyses supported efficacy of IL-6 receptor antagonists.
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| Colchicine | The RECOVERY trial | Randomised, controlled, open-label, platform trial [currently unpublished] | * Preliminary analysis [unpublished] was based on 11,162 randomised patients, 94% of whom were being treated with a corticosteroid. For the primary endpoint of 28-day mortality, there was no significant difference between groups (20% colchicine vs. 19% usual care alone; risk ratio 1.02 [95% confidence interval 0.94-1.11]; p=0.63).
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| Baricitinib (Janus Kinase Inhibitor) | Adaptive COVID-19 Treatment Trial 2 (ACTT-2) | Multinational, randomized, placebo-controlled trial | * Participants (n = 1,033) were randomised 1:1 to oral baricitinib versus placebo in combination with remdesivir.
* Participants receiving baricitinib had a shorter time to recovery than those receiving placebo (median recovery time of 7 vs. 8 days, respectively).
* The effect was most pronounced in those who required high-flow oxygen or non-invasive ventilation (but not invasive ventilation). Difference in mortality between groups was not statistically significant.
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| Low molecular weight heparin | Therapeutic Anticoagulation in Critically Ill Patients with Covid-19 – Preliminary Report (Preprint). The REMAP-CAP, Zarychanski R, ACTIV-4a, ATTACC Investigators | Open-label, adaptive, multi-platform, randomised, clinical trial. | * Therapeutic anticoagulation met pre-defined criteria for futility in patients with severe Covid-19.
* Median organ support-free days were 3 (IQR –1, 16) in patients receiving therapeutic anticoagulation versus 5 days (IQR –1, 16) in patients receiving usual care pharmacological thromboprophylaxis (adjusted odds ratio 0.87, 95% credible interval (CrI) 0.70-1.08, posterior probability of futility [odds ratio<1.2] 99.8%).
* Major bleeding occurred in 3.1% of patients receiving therapeutic anticoagulation and 2.4% of patients receiving usual care thromboprophylaxis.
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| Unfractionated heparin |  |  |  |
| Aspirin | Intermediate‐dose anticoagulation, aspirin, and in‐hospital mortality in COVID‐19: A propensity score‐matched analysis | Retrospective study of hospitalized adult patients with COVID‐19. From March through June 2020, (n=2785).Multivariate regression model. | * For propensity‐score matched patients receiving aspirin (N = 638, in‐hospital aspirin compared to no antiplatelet therapy was associated with a significantly lower cumulative incidence of in‐hospital death (hazard ratio 0.522 [0.336–0.812]).
 |
| Betrixaban (Direct oral anticoagulant) | [APEX Investigators](https://pubmed.ncbi.nlm.nih.gov/?term=APEX+Investigators%5BCorporate+Author%5D) | Randomised control trial | * No significant difference between extended-duration betrixaban and a standard regimen of enoxaparin in primary outcomes of asymptomatic proximal DVT or symptomatic VTE.
* Prespecified exploratory analyses provided evidence suggesting a benefit for betrixaban in the two larger cohorts subsequently analysed.
* No significant different in primary safety outcome: major bleeding, between the groups
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Table I Legend