COVID-19 and immunothrombosis: emerging understanding and clinical management

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Summary

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 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.

Keywords: COVID-19, immunothrombosis, coagulopathy, anticoagulation, immunomodulatory.

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 two 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 prothrombotic 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 consequently microthrombi are formed within small vessels.[3] Interactions between 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 multifaceted 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 II[6] 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

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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.[10-12] Autopsy studies of the lungs of COVID-19 patients demonstrate severe endothelial injury as well as intracellular virus and disrupted cell membranes.[13]

Interestingly, the expression of ACE-2 from the nasal epithelium in particular appears to be age-dependent,[14] 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 'multi-system inflammatory syndrome in children' (MIS-C) around 4–6 weeks after infection.[15,16] In rare cases, some adults have been reported to develop signs and symptoms similar to MIS-C occurring weeks after COVID-19 infection.[17] 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,[18] where underlying endothelial dysfunction is a unifying feature.[19]

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 patients who are critically ill[20] and these complications appear to be occurring despite conventional low-dose venous thromboembolism (VTE) prophylaxis.[21] The most critically ill patients with SARS-CoV-2 infection develop ARDS, with respiratory failure being the leading cause of mortality.[22] Severely ill COVID-19 patients have a median time from onset of dyspnoea to intubation of 10·0 days [interquartile range (IQR) 5·0–12·5 days][23] and median time to intensive care (ICU) admission of 9·5 days (IQR 7·0–12·5 days),[24] 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.[20] 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, four of 123 (3%) were diagnosed with VTE despite receiving thromboprophylaxis.[21]

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,[25,26] the addition of COVID-19 infection exceeds the thrombotic risk which is seen in other respiratory viral illnesses.[21] 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 those with non-COVID-19 ARDS, primarily consisting of pulmonary embolism (11·7% vs 2·1%, $P < 0·008$).[27] Dual-energy CT imaging has been used to examine lung perfusion and investigate for pulmonary embolism/infarction in COVID-19, with some suggesting that scan appearances are more in keeping with pulmonary thrombosis *in situ* rather than pulmonary embolism.[28] Significant difficulties with extracorporeal circuits on machines used in intensive care have also been observed with Helms *et al.* reporting 28 of 29 (96·6%) patients on continuous renal replacement therapy experiencing this complication.[27]

Thrombosis in more unusual sites has been reported in case series of patients with COVID-19 infection, particularly extensive cerebral venous sinus thrombosis as a neurological complication.[29] Although the majority of thrombotic events reported in COVID-19 are venous, arterial events are also recognised, including large-vessel stroke in younger patients. In New York City, over a two-week period from March 23 to April 7 2020, a total of five patients under 50 years old with SARS-CoV-2 infection presented with new-onset symptoms of large-vessel ischaemic stroke; by comparison, every two weeks over the previous 12 months, the same service treated on average 0·73 patients under 50 years with large-vessel strokes.[30] In a cohort from Wuhan, stroke occurred in 2·8% (six out of 214 patients, 41% male, mean age 53 years).[31] Patients with COVID-19 are also at increased risk of other arterial events including mesenteric ischaemia, peripheral vascular ischaemia and myocardial infarction (MI).[32] Acute coronary syndrome can be a complication or a presentation of COVID-19[33] and contributes to the mortality. A large study of 3334 hospitalised COVID-19 patients demonstrated MI was the commonest thrombotic event in their cohort, occurring in 8·9% of patients.[34] 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.[35]

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.[36,37] Diffuse inflammatory alveolar change with macrophage infiltration has been reported in early pathological studies of patients with COVID-19 pneumonia, alongside extensive vascular congestion.[38] Fox *et al.* discovered widespread thrombotic microangiopathy within the pulmonary vasculature and fibrin thrombi were found within distended small vessels/capillaries alongside extensive extracellular fibrin deposition.[39] 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] There is a variation in the microthrombosis rates reported in autopsy studies from between 45% to 100% of COVID-19 patients.[40-42] Carsana *et al.* report platelet-fibrin thrombi in the lung tissue of 87% of patients who died from COVID-19;[41] Wichmann *et al.* also demonstrated pulmonary microthrombi in autopsy lung specimens from COVID-19 patients (n = 12/12), as well as a high incidence of thromboembolic events, including deep vein thrombosis in seven patients (58%) in whom venous thromboembolism was not suspected prior to death, and four cases where pulmonary embolism was the direct cause of death.[42] This highlights thrombosis as a significant contributor to death in COVID-19. Nicolai *et al.* compared histological specimens from the lungs of deceased patients with COVID-19 to those with influenza pneumonia (H1N1 or seasonal). A significantly higher rate of NET formation and immunothrombotic occlusion of microvessels was demonstrated in COVID-19 compared to influenza (40·8% ± 5·4 of vessels affected vs 9·4% ± 4·0 respectively).[43]

In patients with COVID-19 infection, microvascular injury and inflammation can present as a multitude of skin rashes,[44,45] particularly those of a vascular nature such as purpuric lesions.[46] Ischaemic injury of the digits has also been reported in patients with severe COVID-19.[47] 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.[48] Magro *et al.* examined skin as well as 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 interalveolar 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.[36] Moreover, Carvelli *et al.* further demonstrate the significance of complement activation showing that levels of soluble C5a increase in proportion with the severity of illness in patients with COVID-19.[49]

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,[50] with a 2–5-fold elevation in severe COVID-19[51,52] indicating coagulation activation. Other key features of COVID-19 recognised in early data from Wuhan include 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.[53,54] The biochemical features of COVID-19-associated coagulopathy include elevated D-dimer, elevated fibrinogen, mildly prolonged/normal pro-thrombin time and mildly reduced/normal platelet count.[55] 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 significantly higher D-dimer, fibrin degradation products (FDP) levels and a mildly prolonged pro-thrombin time (PT) compared to survivors.[53] 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% vs 43·2%).[56]

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.[53] Mild to moderate thrombocytopenia is also recognised.[23,56] The pattern of parameters in COVID-19-associated coagulopathy certainly has notable differences to the picture of DIC, including generally elevated fibrinogen, preserved platelet count or only mild thrombocytopenia and mild prolongation of PT.[32,55]

Elevated plasminogen has been described in severe COVID-19 infection, 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 assays and higher maximum clot firmness (MCF) in INTEM, EXTEM and FIBTEM assays, thus indicating a severe hypercoagulability rather than consumptive coagulopathy.[57] Significant elevation of Factor V, VIII, and von Willebrand factor (VWF) have also been demonstrated[27] in patients with severe COVID-19 infection. High plasma levels of VWF antigen, as well as VWF pro-peptide, demonstrated in severe COVID-19 are suggestive of endothelial stimulation and damage, with release of VWF from Weibel–Palade bodies.[58,59]

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 activated partial thromboplastin time (aPTT).[60] Anticardiolipin and B2GP1 IgG or IgM antibodies

have been shown to be present in 10% of hospitalised patients in a study of 50 patients[61] but it remains unclear whether these antibodies are persistent or transient in nature as is common with other infections.[62]

As highlighted in the early studies from China, lactate dehydrogenase (LDH) — an indicator of cell death — was found to be significantly more elevated in patients admitted to ICU *versus* those who did not require critical care (400 vs 281 U/l, $P = 0{\cdot}0044$).[50] Likewise, LDH is significantly more elevated in severe COVID-19 infection vs non-severe infection (424 vs 204 U/l, $P < 0{\cdot}001$).[63] Lymphopenia is commonly observed in tandem to suggest immune cell death and its severity correlates with more severe disease requiring intensive care/ventilatory support and in non-survivors.[50,52,56,63,64,65] 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.[64-66]

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.[67] Huang *et al.* first reported that D-dimer was fivefold higher than the normal range in severe COVID-19 in 41 patients hospitalised in Wuhan, China[50] and elevated D-dimer was an independent predictor for mortality in hospitalised patients with COVID-19.[53,68] Zhou *et al.* proposed a D-dimer value of 1 μg/ml for predicting mortality,[23] 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 value[69] vs 2·0 μg/ml to predict hospital mortality [hazard ratio (HR) 51·5, 95% confidence interval (CI):12·9–206·7].[68]

Potential drivers of immunothrombosis in COVID-19

Thrombosis as a component of the innate immune response against pathogens is called immunothrombosis[3] and can be a potentially fatal complication of infection.[70] 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.[71] 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 formation of NETs to promote immunothrombosis.[72,73] 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.[73,74]

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.[75] Furthermore, there is endothelial dysfunction including increased expression of adhesion molecules and increased vascular permeability which in turn contributes to a pro-thrombotic state.[75,76] TF expression is increased following pro-inflammatory cytokine release and acquired protein C deficiency develops due to a combination of consumption, increased degradation and reduced synthesis.[77,78] 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.[79,80]

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.[81,82]

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.[50] In general terms, viral RNA has been shown to activate Toll-like receptors (TLR)-3 and -7 to enhance the NF-κB pathway and the interferon regulatory factors (IRFs), which consequently increases the synthesis and release of pro-inflammatory cytokines.[83] We know that elevated IL-6 stimulates coagulation by increasing thrombin generation[84] and anti-IL-6 antibody tocilizumab has shown beneficial effects when it was used in COVID-19 to target both inflammation and coagulation.[85,86] Elevated IL-1, IL-6, and TNFα could activate endothelial cells to promote thrombosis[80,87] 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 TF to promote fibrin deposition and thrombosis.[88]

Elevated IL-8 increases neutrophil recruitment and has been demonstrated to induce formation of NETs,[89,90] which form a scaffold to promote thrombosis.[91] 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.[90] Elevated levels of myeloperoxidase as a NET-related marker[92] have been shown in the sera of COVID-19 patients, and Barnes *et al.* showed infiltration of

neutrophils in COVID-19 autopsy specimens.[93] Middleton *et al*. subsequently demonstrated NET-containing microthrombi with neutrophil and platelet infiltration in pulmonary autopsy samples.[94] 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.[95] 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.[74,96] Our recent data demonstrate that circulating histones on admission to hospital were significantly elevated with increasing severity of COVID-19 infection [Mild, median = 2·6 μg/ml (IQR = 0·7–7.6), Moderate, 10·5 μg/ml (3·5–27.2), Critical, 20·0 μg/ml (6·2–33.0), Non-survivors, 29·6 μg/ml (11·2–60.0); $P < 0.001$].[97] 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.[97]

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 (Fig. 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, pro-inflammatory 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 pro-coagulant[96] and cytotoxic and mediate multiorgan injury.[98] We propose that these factors act together to initiate and amplify the activation of coagulation, fibrin deposition and immunothrombosis.

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) were the first to demonstrate a significant reduction in 28-day mortality amongst hospitalised patients with COVID-19, requiring either mechanical ventilation or oxygen alone, who received dexamethasone 6 mg for 10 days. Regarding 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 ($P = 0·002$).[99] 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 seven days of fixed-dose hydrocortisone (or 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.[100] 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 HR of 1·61 when compared to control group (95% credible interval, 1·25–2·08). The median number of organ-support-free days with tocilizumab was 10 days (IQR −1 to 16), with sarilumab, 11 days (IQR 0–16) and 0 days in the control group (IQR −1 to 15).[86] 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; odds ratio (OR) 1·64; 95% credible interval, 1·25–2·14; Table I).[85] 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 [three days (IQR –1, 16) vs 5 days (IQR –1, 16; adjusted OR 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

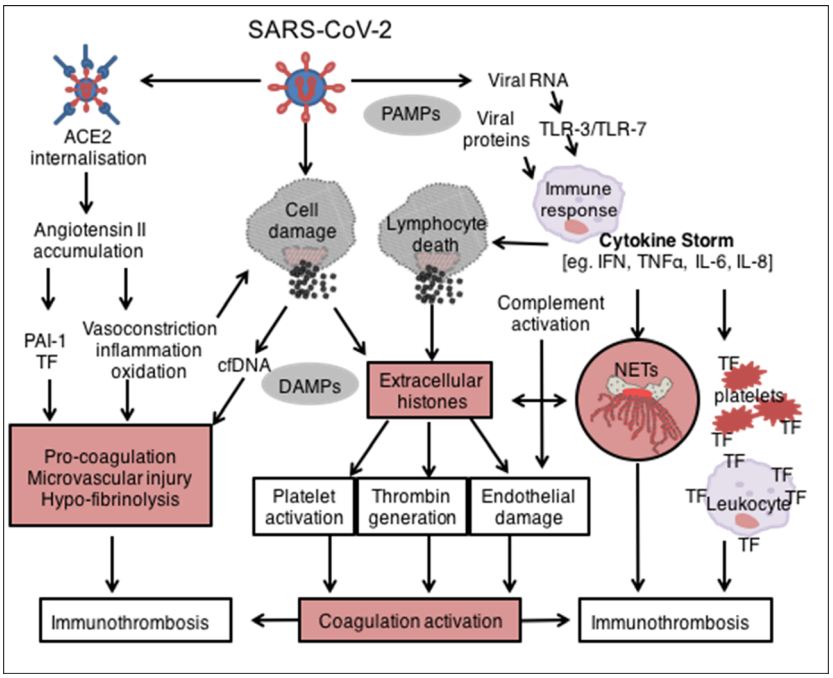

Fig 1. Mechanisms underlying immunothrombosis in COVID-19. TF, tissue factor; TLR, Toll-like receptors; cfDNA, cell-free DNA; DAMPs, damage-associated molecular patterns; IFN, interferon; NETs, neutrophil extracellular traps; PAI-1, plasminogen activator inhibitor-1; PAMPs, pathogen-associated molecular patterns; TNFα, tumour necrosis factor α. [Colour figure can be viewed at wileyonlinelibrary.com]

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 vs 2·4% of patients receiving standard care thromboprophylaxis.[101] Further to this, recent results from the INSPIRATION randomised clinical trial showed that for COVID-19 patients admitted to ICU (n = 562), 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. Major bleeding events were reported in 2·5% of the intermediate-dose group vs 1·4% of the standard-dose prophylaxis group, which did not meet the prespecified criteria for non-inferiority (P > 0·99). Clinically relevant non-major bleeding events were reported in 4·3% of the intermediate-dose group vs 1·7% in the standard-dose prophylaxis group [OR 2·55 (95% CI, 0·92–7·04); P = 0·07]. In the intermediate-dose group, one case of intracranial haemorrhage and two fatal bleeding episodes were reported. A major limitation of this study includes the dosing in the intermediate group being 1mg/kg once daily (as opposed to a lower dose twice daily) and the severity of the cohort studied was less severe than is typical of ICU cohorts elsewhere.[102] Currently, observational data suggest a benefit of intermediate dose heparin but data from larger patient numbers in randomised control trials would be beneficial to determine optimal dosing of anticoagulation.[103,104]

In addition to anticoagulation, heparin also has anti-inflammatory effects[105] and has even shown potential anti-viral effects in pre-clinical trials in COVID-19.[106] Heparin also acts to prevent histone-mediated cytotoxicity and has been shown to improve survival in sepsis.[107] 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]).[103] These findings are reflected in an observational cohort study in the United States demonstrating a reduced in-hospital mortality (adjusted HR, 0·53, 95% CI, 0·31–0·90, P = 0·02), and a reduced risk of mechanical ventilation for those patients taking aspirin (adjusted HR, 0·56, 95% CI, 0·37–0·85, P = 0·007).[108] Results regarding aspirin from the RECOVERY randomised study of more than 14 000 participants are keenly awaited, and aspirin as well as P2Y12 inhibitors are currently being trialled in the REMAP-CAP and ACTIV4 studies.[109,110]

Pulmonary embolism, particularly multiple, small pulmonary vascular occlusions were commonly found at autopsy and significantly contributed to ARDS and mortality.[42] These patients were usually critically unwell or had

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)	Multicentre randomised open-label adaptive trial in hospitalised patients with suspected/confirmed COVID-19 (n = 6 425). United Kingdom	28-day mortality 22·9% in the dexamethasone arm vs 25·7% in the 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 the dexamethasone arm vs 41·4% in the 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, multinational, 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
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 mechanically ventilated at baseline, patients receiving tocilizumab were less likely to reach the composite end-point 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 vs 0 (IQR −1, 15) for control Hospital mortality was 28·0% (98/350) for tocilizumab vs 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 vs 0 (IQR −1, 15) for control respectively Hospital mortality was 22·2% (10/45) for sarilumab vs 35·8% (142/397) for control group All secondary outcomes and analyses supported efficacy of IL-6 receptor antagonists
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 end-point of 28-day mortality, there was no significant difference between groups [20% colchicine vs 19% usual care alone; risk ratio 1·02 (95% CI 0·94–1·11); $P = 0·63$]
Baricitinib (Janus Kinase Inhibitor)	Adaptive COVID-19 Treatment Trial 2 (ACTT-2)	Multinational, randomized, placebo-controlled trial	Participants (n = 1033) 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
Baricitinib (Janus Kinase Inhibitor)	COV-BARRIER study	Randomized, double-blind, placebo-controlled study (n = 1 525; press release)	Did not meet statistical significance on primary end-point of progression to non-invasive ventilation or invasive mechanical ventilation or death (press release) 38% reduction in mortality by D28 ($P = 0·0018$) in patients treated with baricitinib in addition to standard of care (including corticosteroids and remdesivir)

Table I. (*Continued*)

Treatment	Study group	Study population & sample size	Study summary
Heparin (Unfractionated and low molecular weight)	Therapeutic anticoagulation in critically ill patients with Covid-19 – preliminary report (Preprint). The REMAP-CAP, Zarychanski R, ACTIV-4a, ATTACC Investigators	Open-label, adaptive, multiplatform, 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 vs 5 days (IQR −1, 16) in patients receiving usual-care pharmacological thromboprophylaxis [adjusted odds ratio 0·87, 95% CI 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
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 = 2 785) 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 [HR 0·522 (0·336–0·812)]

CI, confidence interval; HR, hazard ratio; OR, odds ratio.

shown a sudden deterioration and thereby required intensive care. As discussed, computed-tomography pulmonary angiogram (CTPA) was used for detecting pulmonary thrombosis/embolism in COVID-19 patients. 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 emerging in COVID-19, and there appears to be a low reported incidence of major bleeding[111] or DIC with bleeding events;[27,53] only a small fraction of COVID-19 patients truly developed DIC or overt bleeding, but these were strongly associated with mortality.[54] Fibrin deposition in the pulmonary microvasculature is considered as a contributory cause of ARDS,[112] 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.[110] A multitude of clinical trials for COVID-19 therapies targeting inflammation and thrombosis are under way globally, the ACTIV (Accelerating COVID-19 Therapeutic Interventions and Vaccines) trials alone are currently studying immune modulators (infliximab, abatacept and cenicriviroc), a novel 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.[109]

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.[113] Further studies to explore blocking upstream of coagulopathy, such as inhibiting immune cell death or neutralising 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.[114] Vechi *et al.* report a case series of thrombotic events occurring 3–4 weeks following recovery and apparent clinical improvement from COVID-19.[115] 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 anticipated.[116]

In the meantime, the world's focus remains on the COVID-19 vaccination roll-out. More than one 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 immunologically mediated 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 vaccinated and is affecting patients of all ages and both genders.[117] The syndrome has been reported in around 250 cases in the UK and consists of venous thrombosis (often in unusual sites including cerebral sinus venous thrombosis and splanchnic vein thrombosis) with concurrent thrombocytopenia occurring around 5–14 days following a first dose of ChAdOx1 nCoV-19 adenoviral vector vaccine.[118-120] 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.[118] Current expert advice in the UK recommends treatment with intravenous immunoglobulin, to anticoagulate with non-heparin anticoagulants and consider plasma exchange therapy in very severe or resistant cases,[121] but these management guidelines may change as further evidence emerges.

The spate of data, experiments and insights have 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 the manuscript; CB wrote the management section and critically reviewed the manuscript; STA and GW designed Fig. 1, wrote the drivers of immunothrombosis section and critically reviewed the manuscript; CHT provided overall supervision, wrote and critically reviewed the manuscript.

Conflicts of interest

All the authors have completed an ICJME conflict of interest disclosure form and have no conflicts of interests to declare.

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