




Targeting coagulation activation in severe COVID-19 pneumonia: lessons from bacterial pneumonia and sepsis

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A large armamentarium of potentially beneficial anticoagulant agents exists, and well-designed randomised clinical trials are now needed to investigate the wide range of anticoagulant and anti-fibrinolytic therapies to determine the optimal strategy <https://bit.ly/2CI459q>

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ABSTRACT Novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), has rapidly spread throughout the world, resulting in a pandemic with high mortality. There are no effective treatments for the management of severe COVID-19 and current therapeutic trials are focused on antiviral therapy and attenuation of hyper-inflammation with anti-cytokine therapy. Severe COVID-19 pneumonia shares some pathological similarities with severe bacterial pneumonia and sepsis. In particular, it disrupts the haemostatic balance, which results in a procoagulant state locally in the lungs and systemically. This culminates in the formation of microthrombi, disseminated intravascular coagulation and multi-organ failure. The deleterious effects of exaggerated inflammatory responses and activation of coagulation have been investigated in bacterial pneumonia and sepsis and there is recognition that although these pathways are important for the host immune response to pathogens, they can lead to bystander tissue injury and are negatively associated with survival. In the past two decades, evidence from preclinical studies has led to the emergence of potential anticoagulant therapeutic strategies for the treatment of patients with pneumonia, sepsis and acute respiratory distress syndrome, and some of these anticoagulant approaches have been trialled in humans. Here, we review the evidence from preclinical studies and clinical trials of anticoagulant treatment strategies in bacterial pneumonia and sepsis, and discuss the importance of these findings in the context of COVID-19.

Introduction

Novel coronavirus disease 2019 (COVID-19) first emerged in Wuhan, China, and has rapidly spread across the globe. COVID-19 is caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and, as of August 7, 2020, there have been ~19 million reported cases and 700 thousand deaths [1]. The only treatment thus far to demonstrate a reduction in mortality of severe COVID-19 is dexamethasone [2]. The antiviral remdesivir has been shown to reduce hospital length of stay [3]. Therefore, identifying successful therapeutic strategies still remains a major ongoing challenge.

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The pathogenesis of severe COVID-19 pneumonia has similarities with that of severe bacterial pneumonia, with both causing severe hypoxia that often requires ventilatory support. Both are characterised by extensive inflammatory cell recruitment to the lungs, a potent acute phase reaction and raised levels of pro-inflammatory cytokines (figure 1) [4, 5]. Severe COVID-19 pneumonia and severe bacterial pneumonia are both associated with widespread activation of the coagulation system, evidenced by elevated activated partial thromboplastin time (APPT) and prothrombin time (PT) and markedly elevated D-dimer levels, with raised levels of the latter being associated with poor prognosis [6–9]. Evidence of disseminated intravascular coagulation is seen frequently in patients at increased risk of death from COVID-19 [8]. In severe COVID-19, the clinical relevance of activation of the coagulation system is evidenced by a high incidence of pulmonary and peripheral venous thromboembolic (VTE) disease, stroke and acute coronary syndromes, even in patients receiving prophylactic heparin [8, 10, 11]. The development of microthrombi in lung capillaries [12] may partially explain the profound hypoxia seen in some patients with COVID-19, and similar microthrombi may contribute to renal and cardiac involvement. Recent studies suggest that the rapid accumulation of neutrophil extracellular traps in the microvessels of patients with severe COVID-19 results in occlusion of these vessels and promotes microthrombosis [13].

Although endothelial dysfunction occurs in bacterial pneumonia and sepsis, it is pronounced in COVID-19, with SARS-CoV-2 having a direct effect on the endothelium. *Post mortem* studies have shown evidence of intracellular virus and microangiopathy [12]. SARS-CoV-2 uses the angiotensin-converting enzyme (ACE)-2 receptor to enter the host cells and activates the renin–angiotensin system, which can induce a prothrombotic state [14]. Furthermore, the host inflammatory response to the virus promotes activation of coagulation and reduced fibrinolysis. Older age, low lymphocyte count, prolonged PT and APPT, and admission to the intensive care unit have been identified as risk factors for thrombosis [15].

COVID-19 pneumonia is characterised by increases in multiple cytokines, including tumor necrosis factor (TNF) and interleukin-6 (IL-6) [16], which are potent activators of the tissue factor (TF)-dependent coagulation cascade [17–19]. Activation of the coagulation system is known to be pro-inflammatory and could drive further increases in inflammation; in tissues with a delicate architecture such as the distal lung, this can be highly detrimental, impairing gas exchange [20] and culminating in acute respiratory distress syndrome (ARDS) [21, 22]. The extensive activation of coagulation in patients with severe COVID-19 could stimulate further inflammation *via* the mechanisms described below, resulting in a positive feedback loop that maintains high levels of inflammation for a prolonged period. Hence, effective anticoagulation strategies may prevent complications associated with aberrant clotting, attenuate coagulation-induced exaggerated inflammatory responses and potentially reduce the severity and extent of pulmonary infiltrates.

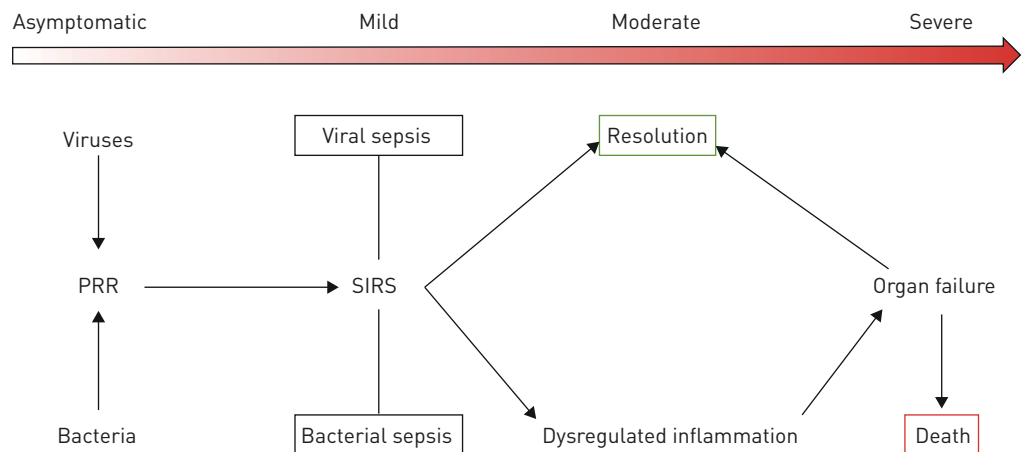


FIGURE 1 Similarity in progression of severe viral and bacterial pneumonia. During viral and bacterial infection, the pathogens are recognised by host pathogen recognition receptors (PRR) on the surface of epithelial cells, mononuclear phagocytes and other cell types, resulting in initiation of various inflammatory cascades triggering systemic inflammatory response syndrome (SIRS). At this stage patients will have viral/bacterial sepsis with life-threatening organ dysfunction. Most individuals will clear the pathogen (with antimicrobial/antiviral therapy) and the body resolves the inflammation. Others, however, will not be able to clear the pathogen, or despite clearance of the pathogen will continue to exhibit dysregulated inflammatory responses that are unabated, resulting in tissue injury and organ failure. Of these patients, some receiving supportive care will recover and some will die.

Although less clinically evident than in COVID-19, severe bacterial pneumonia and sepsis are also associated with activation of coagulation locally in the lungs and systemically, and are associated with bystander tissue injury and correlate negatively with survival [9, 23, 24]. As a consequence, therapies that modulate inflammatory responses caused by activation of coagulation have been extensively investigated in bacterial pneumonia and sepsis. Several anticoagulant therapeutic strategies for treating pneumonia, sepsis and ARDS have been identified and some of these anticoagulants have been trialled in humans. The high frequency of coagulation abnormalities and prolonged inflammation that occurs in severe COVID-19 suggests these therapies are attractive potential therapies.

Here, we review the evidence from preclinical studies and clinical trials of anticoagulant treatment strategies in bacterial pneumonia and sepsis and discuss how these data could be relevant for managing COVID-19 pneumonia. The data will be discussed in relation to 1) activation of the TF–thrombin generation pathway, 2) endogenous anticoagulants and 3) coagulation–inflammation crosstalk (figure 2).

The TF-dependent pathway

The TF pathway is the main initiator of procoagulant activity secondary to tissue injury and inflammation, and it plays an important role in pneumonia and ARDS [25, 26]. TF is a transmembrane glycoprotein expressed by mononuclear cells, endothelial cells, fibroblasts, vascular smooth muscle cells and alveolar epithelial cells but it can also be detected in extracellular fluids and in cell-derived microparticles shed from activated cells [27–29]. Under normal physiological conditions, TF will only come into contact with blood or circulating coagulation zymogens after vascular injury and disruption of the endothelial barrier [30, 31]. TF binds to and activates coagulation factors VII to VIIa, forming the TF–VIIa complex. This initiates the TF-dependent pathway of coagulation by activating coagulation factor X to Xa and then binding factor Xa to form the TF–VIIa–Xa ternary complex. The TF–VIIa–Xa ternary complex converts prothrombin (factor II) to thrombin (factor IIa), a process that is sustained and increased in efficiency by positive feedback through activation of coagulation factors V and VIII, which are non-enzymatic cofactors that activate factor X [32, 33]. Thrombin converts fibrinogen to fibrin, the main constituent of clots, and is also the main physiological activator of proteinase-activated receptor-1 (PAR₁), the major high-affinity thrombin receptor that promotes multiple downstream cellular responses involved in tissue repair. However, when dysregulated, these cellular responses promote inflammation and lead to disruption of the epithelial and endothelial barriers and the development of fibrosis in multiple organs, including the lung, kidney and liver [34], thereby contributing to disease pathogenesis during severe infections.

TF pathway inhibitors

TF and factor VIIa levels are elevated in the bronchoalveolar lavage fluid of patients with severe pneumonia, and the central role of TF for initiating coagulation makes it an attractive target for therapeutic intervention against the potential negative consequences of coagulation activation. Tissue factor pathway inhibitor (TFPI) is a central endogenous regulator of TF pathway activity and thrombin generation. This glycoprotein is mainly expressed by endothelial cells and platelets and acts by directly inhibiting factor Xa. The Xa–TFPI complex subsequently also inhibits the TF–VIIa complex. In preclinical non-human primate models of endotoxaemia, blockade of TF–VIIa, using a competitive inhibitor of TF (site-inactivated factor VIIa), inhibits the TF pathway and reduces lung inflammation and deposition of fibrin [35, 36]. A phase II clinical trial of recombinant human TFPI (rhTFPI; Tifacogin) in patients with severe sepsis decreased concentrations of IL-6, an important pro-inflammatory cytokine that is increased in COVID-19 pneumonia and a major target in ongoing clinical trials [37, 38]. However, in the Optimised Phase III Tifacogin (rTFPI) in Multicenter International Sepsis Trial (OPTIMIST phase III) for patients with severe sepsis with a high international normalised ratio (≥ 1.2), rhTFPI did not improve 28-day mortality, despite reducing evidence of inflammation [39]. Administration of rhTFPI and heparin concomitantly was associated with increased bleeding, although a *post hoc* analysis did suggest rhTFPI improved survival in patients with severe community-acquired pneumonia (CAP) for whom the microbiological aetiology was known and heparin had not been given [40]. However, the subsequent Recombinant Tissue Factor Pathway Inhibitor in Severe Community-Acquired Pneumonia (CAPTIVATE) trial [41] did not demonstrate improved survival for patients with severe CAP treated with rhTFPI. Although TFPI was largely disappointing in the setting of sepsis, the advantages of targeting the TF pathway in preventing the development of capillary microthrombi, as well as inflammation, might outweigh potential risks.

Endogenous anticoagulants and treatment of severe pneumonia and ARDS

Activation of the coagulation system is countered by endogenous anticoagulants, which act either alone or in concert to limit coagulation. A number of endogenous anticoagulants, including anti-thrombin (AT) and activated protein C (APC), have been trialled in severe pneumonia and ARDS, on the basis that they

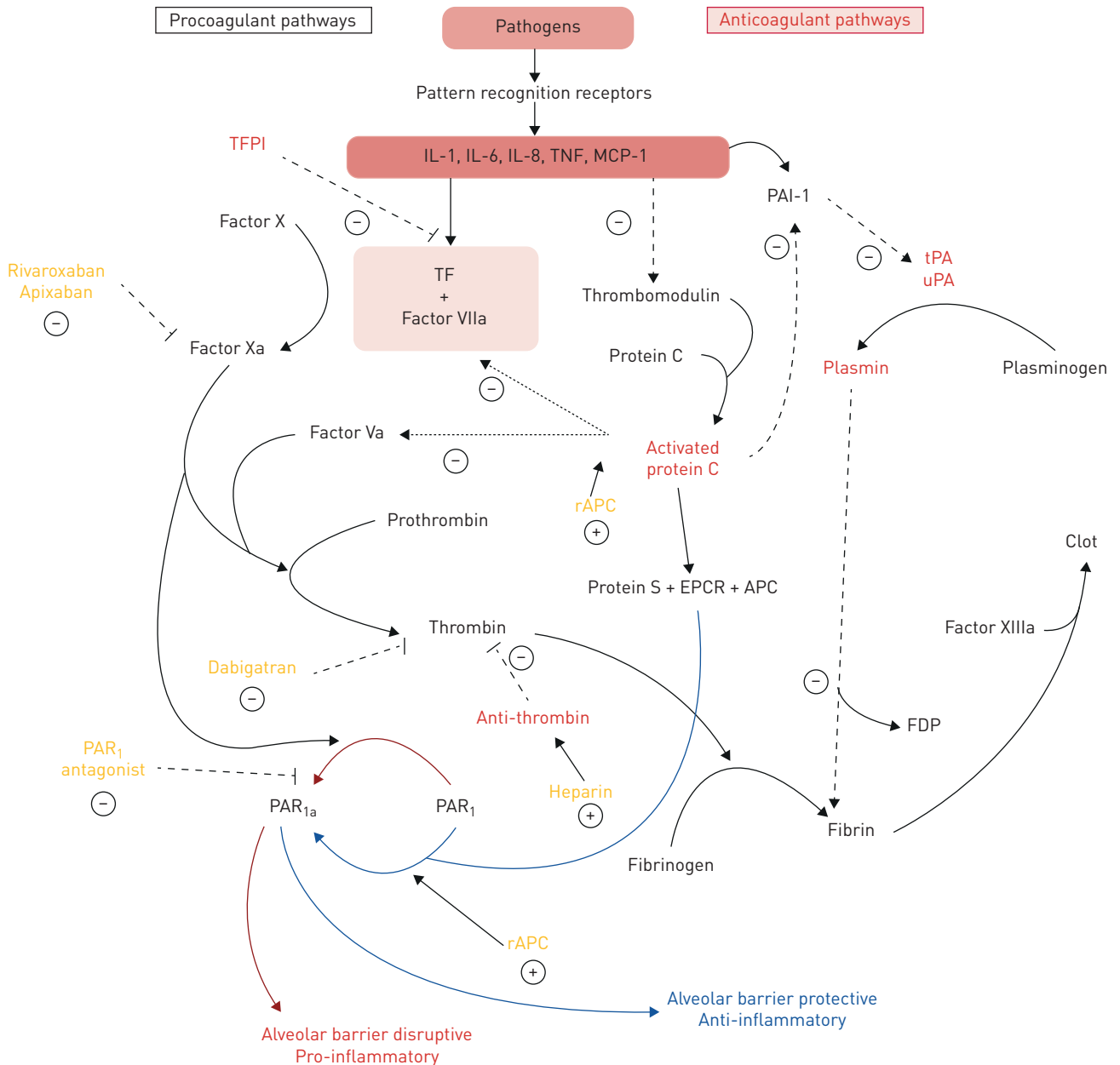


FIGURE 2 Activation of the coagulation cascade and endogenous anticoagulants. Pathogens in the lungs are recognised by pattern recognition receptors that initiate a pro-inflammatory response and expression of tissue factor (TF) allowing factor VIIa to come in to contact with TF. TF-VIIa activates factor X that binds to TF-VIIa to form a ternary complex that converts prothrombin to thrombin (factor IIa). Factor Va binds to the ternary complex to form the prothrombinase complex and to platelets and factor Xa to form the major prothrombinase complex that generates the large amounts of thrombin necessary for fibrin clot formation. Thrombin also increases expression of thrombomodulin on endothelial cells and activates proteinase-activated receptor-1 (PAR₁), the major thrombin receptor. Activation of PAR₁ by thrombin leads to downstream signalling that is pro-inflammatory and leads to disruption of the alveolar barrier (brown). Activation of PAR₁ by activated protein C (APC) leads to signalling that has barrier-protective and anti-inflammatory effects (blue). TFPI: tissue factor pathway inhibitor; IL: interleukin; TNF: tumor necrosis factor; MCP: monocyte chemoattractant protein; PAI: plasminogen activator inhibitors; tPA: tissue-type plasminogen activator; uPA: urokinase-type plasminogen activator; EPCR: endothelial protein C receptor; FDP: fibrin degradation products.

were shown to be protective against lung injury in experimental animal models by reducing fibrin generation and attenuating neutrophil recruitment [42–46].

Anti-thrombin

AT inactivates thrombin and factor Xa and has been shown to reduce systemic (after intravenous administration) and bronchoalveolar (after both intravenous and nebulised administration) thrombin and fibrin levels and reduce lung injury in preclinical models [42, 46]. However, the clinical data currently do not support using AT as a treatment to improve lung injury. The KyperSept trial of high-dose AT

treatment in severe sepsis [47] showed no effect on 28-day mortality, and an increased risk of bleeding events in the AT-treated participants was observed, particularly in those who received concomitant heparin. Yet, a subgroup analysis revealed that the AT-treated group that had not received concomitant heparin had a 15% improvement in 90-day mortality compared to those treated with heparin. Nonetheless, a meta-analysis of 20 trials of a heterogeneous population of critically ill patients concluded that AT should be avoided owing to the risk of bleeding complications [45]. Natural or pharmacological thrombin inhibitors have not been tested in clinical trials in the setting of pneumonia and potential clinical translation has been inferred from studies of AT in sepsis, in which pneumonia is the leading cause in a large proportion of cases. In terms of extrapolation to COVID-19 pneumonia, we would propose that the current evidence would also argue against the use of AT, particularly if co-administered with heparin. Heparin alone, which exerts its anticoagulant effects *via* the activation of AT, is currently being evaluated in multiple trials in the context of COVID-19 pneumonia. This includes evaluation of a systemic prophylactic dose and a full therapeutic dose of low molecular weight heparin (LMWH). In H1N1 ARDS, anticoagulation with systemic heparin significantly reduced the high incidence of VTE [48]. Heparin also exerts anti-inflammatory and antiviral properties [49, 50]. However, the route of administration of heparin is likely to be critical. Nebulised heparin did not attenuate inflammation in a murine model of pneumonia [42], and, in clinical trials in patients with or at risk of ARDS, nebulised heparin had no major impact on physiological variables nor a beneficial effect on mortality, although it increased ventilator-free days [51–53].

Activated protein C

APC plays a critical role in terminating coagulation by proteolytically inactivating factors Va and VIIIa, and has been widely investigated in the context of sepsis [54–56]. Once the coagulation cascade is activated, thrombin binds to thrombomodulin and, facilitated by the endothelial protein C receptor (EPCR), protein C is activated. APC then binds to the EPCR and, together with its co-factor protein S, forms the EPCR-APC-protein S complex that binds and degrades factor Va and factor VIIIa [57], providing negative feedback for thrombin generation. APC also exerts pro-fibrinolytic and anti-inflammatory effects [57, 58]. Preclinical studies support a beneficial effect of APC administration, showing that rhAPC attenuates tissue injury and improves survival in models of sepsis and lung injury [59–61]. For example, in murine models of indirect lung injury (intravenous injection of lipopolysaccharide) and direct lung injury (*Streptococcus pneumoniae* infection), nebulised rhAPC specifically reduced local bronchoalveolar thrombin and fibrin generation without affecting intravascular thrombin generation or fibrinolytic activity [46] and without adversely affecting bacterial clearance [46]. This provides proof-of-principle that targeting the alveolar epithelium locally with nebulised rhAPC can attenuate coagulation activation without compromising host defence.

In clinical studies, systemic, rather than nebulised, delivery of APC has had initially promising results. In the original landmark PROWESS study, intravenous infusion of rhAPC (drotrecogin alpha (activated) (DrotAA)) significantly reduced 28-day all-cause mortality in patients with sepsis (secondary to CAP) by 28% and reduced the resolution time of respiratory failure [22]. This resulted in the Infectious Diseases Society of America/American Thoracic Society recommending APC for the treatment of refractory septic shock due to CAP [62]. Although APC reduced coagulation activation and lung injury scores in a study of 27 patients with ARDS [63, 64], in a subsequent randomised controlled trial APC did not improve the clinical outcomes of ARDS patients [65] or of patients with sepsis and low of risk of death [66]. Furthermore, a meta-analysis of five studies involving 5101 participants concluded that APC was associated with higher risk of bleeding and should not be used in patients with severe sepsis or septic shock [67]. Importantly, the PROWESS-Shock study [55] failed to demonstrate an improvement in survival in patients with septic shock and eventually led to the withdrawal of DrotAA (Xigris) from the market. However, the significant differences in patient characteristics between the PROWESS trials has led to the recommendation that a trial of DrotAA should be repeated using an optimised study design [68], and this could include a trial in a less heterogeneous high-risk population, such as COVID-19 pneumonia. The preclinical and clinical data suggest nebulised rhAPC may not carry the same bleeding liability as intravenous administration and may be an appropriate route of drug administration. Alternatively, the bleeding risk could be mitigated using an rhAPC variant with <10% anticoagulant activity [69], which in preclinical studies was as effective as wild-type APC in improving survival of mice in sepsis models [69].

In a small study of 11 COVID-19 patients, most had increased endogenous APC levels [70]; however, four patients had lower APC levels and in this subgroup of patients, particularly those with septic shock or at high risk of death, administration of rhAPC might warrant further investigation.

Endogenous fibrinolytics

Fibrin deposition and clearance is regulated by the control of plasmin activity, which in turn is regulated by the relative balance between plasminogen activators and plasminogen activator inhibitors (PAI)-1, -2

and -3. Plasminogen is mainly synthesised in the liver and converted into plasmin by the serine proteinases tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). Several preclinical and clinical studies have demonstrated elevated levels of PAI-1 in pneumonia [30, 71, 72]. However, preclinical studies on the effects of PAI-1, tPA and uPA in murine models of pneumonia suggest the outcome is pathogen dependent. For example, in a *Klebsiella pneumoniae* pneumonia model, PAI-1 improved bacterial clearance and reduced mortality [73], but neither PAI-1 nor plasmin appeared to have a role in mouse models of *S. pneumoniae* [72] or *Pseudomonas aeruginosa* pneumonia [74]. Instead, the urokinase plasminogen activator receptor axis promoted host defence against *S. pneumoniae* by recruiting neutrophils to the alveoli and enhancing neutrophil-mediated bacterial killing [75]. Furthermore, in a model of sterile lung injury, tPA administration reduced alveolar leak but had no effect on pulmonary inflammation [76]. Data are limited regarding the role of this system in viral respiratory infection models but in a study of influenza A infection, the absence of plasminogen reduced inflammation [77]. A recent meta-analysis of preclinical studies of fibrinolytics in acute lung injury suggested increased fibrinolysis, attenuated inflammation and alveolar leak, and improved survival [78].

There are limited clinical data on the efficacy of targeting fibrinolysis in patients with severe infections or ARDS. However, patients with ARDS display evidence of a marked reduction in fibrinolysis, with evidence of reduced uPA activity and increased PAI-1 levels in bronchoalveolar lavage fluid [30]. Furthermore, one study demonstrated a shorter length of stay in the intensive care unit and improved survival in ARDS patients treated with nebulised streptokinase [79]; this pathway may represent an interesting target for the management of COVID-19-induced lung injury. Two trials targeting this pathway are currently underway, one in the UK (nebulised r-tPA) and another in the USA (alteplase) (NCT04356833, NCT04357730).

PARs mediate the interplay between coagulation and inflammation

The discovery of the PARs in 1991 represented a watershed moment in our understanding of the mechanism by which coagulation proteinases directly influence cell function. PARs are seven transmembrane domain G-protein-coupled receptors that have unique mechanisms of activation, which involves limited proteolysis of their amino-terminal exodomains to unmask a tethered ligand. Four PARs (PAR₁₋₄) are differentially expressed on lung-resident cells, including lung epithelial cells, endothelial cells and fibroblasts, as well as on recruited monocytes and neutrophils following tissue injury. Thrombin is a major activator of PAR₁ and PAR₃, whereas factor Xa and the potent TF-Xa-VIIa ternary complex activate PAR₁ and PAR₂ [80]. PAR₁ can also be activated by the EPCR-APC complex [81], plasmin [82] and matrix metalloprotease-1 [83], whereas trypsin and tryptase can activate PAR₂ [80].

Activation of PAR₁ induces the expression of a host of inflammatory cytokines, chemokines and growth factors that influence inflammatory cell trafficking, leukocyte activation and endothelial permeability [84, 85]. However, PAR₁ downstream signalling is highly context dependent and influenced by both the nature and the extracellular concentration of activating proteinases [86]. For example, *in vitro* studies using exogenously added proteinases have shown that thrombin increases vascular permeability *via* PAR₁ activation and coupled signalling of the S1P3 receptor [87]. Conversely, APC inhibits thrombin-mediated vascular hyper-permeability *via* PAR₁ activation and signalling *via* the S1P1 receptor [86, 88]. During infection the deleterious thrombin-PAR₁ signalling responses appear to predominate over protective APC-PAR₁ signalling responses because endogenous APC levels are depleted and APC has 500 times lower affinity and efficiency for cleaving PAR₁ compared to thrombin [89, 90]. The differential effects of PAR-1 activation by APC or thrombin are also explained by differential cleavage of the PAR₁ N-terminus. Thrombin cleaves PAR₁ at Arg41, whereas APC cleaves PAR₁ at Arg41 and Arg46, with preferential cleavage at the latter site mediating the cytoprotective effects of APC [91, 92]. Hence, in the presence of increased thrombin generation, as often seen in severe pneumonia, the beneficial effects of low levels of endogenous APC may be overcome by the barrier-disruptive effects of thrombin-induced PAR₁ cleavage.

In preclinical studies, survival from sepsis appears to be related to the time of activation of PAR₁ [93]; mice treated early with a PAR₁ antagonist were protected against thrombocytopenia and had reduced thrombin levels and improved survival [93]. *S. pneumoniae* pneumonia in PAR₁-knockout mice exhibited evidence of reduced lung injury and neutrophil recruitment without any effect on bacterial clearance [94], whereas the PAR₁ antagonist vorapaxar reduced neutrophilic inflammation; TNF, IL-1 β , C-C motif chemokine ligand 2 (CCL2) and CCL7 levels; coagulation activation; and vascular permeability (alveolar leak) without adversely affecting bacterial clearance. Murine studies of viral infection, including influenza [95, 96], suggest PAR₁ is required for host control of virus load initially, but if viral replication is left unrestricted PAR₁ promotes inflammation and increases mortality. These preclinical findings support the rationale that blockade of PAR₁ may be beneficial in attenuating pathogen-induced hyper-inflammatory responses and in maintaining integrity of the alveolar endothelial barrier, but that the benefits of this treatment may differ with the timing of treatment and the causative pathogen.

The potential of targeting coagulation in COVID-19

As previously highlighted, severe COVID-19 is associated with overwhelming clinical complications of coagulation activation. A retrospective study of 107 patients who had received at least 1 month of anticoagulation therapy prior to SARS-COV-2 infection demonstrated that none developed clinically relevant thrombotic complications [97]. Prophylactic dose heparin is therefore recommended for all patients admitted to hospital with COVID-19 [98]. The enhanced inhibition of thrombin with higher doses of LMWH may further benefit patients by subsequently reducing the downstream signalling involved in inflammation, although the bleeding risk would need to be carefully considered. Clinical trials of low dose *versus* full dose LMWH are currently underway (NCT04372589, NCT04401293, NCT04367831, NCT04345848, NCT04373707, NCT04366960, NCT04359277 and NCT04397510) and will inform the future clinical management of COVID-19. However, in the context of diseases with a profound inflammatory response and potential lower levels of AT such as COVID-19 [8, 70], LMWH may not be effective. Indeed, anecdotal observations from clinicians caring for patients with COVID-19 report that patients continue to develop clinically identifiable clots, despite receiving prophylactic doses of LMWH, and that higher doses of heparin are needed according to factor Xa analysis, especially in patients with significantly elevated levels of D-dimer [98]. This suggests that it may be important to target coagulation proteinases that do not rely on the presence of circulating endogenous anticoagulants. In this context, some reports suggest that bivalirudin may improve haemofilter and extracorporeal membrane oxygenation filter survival [99, 100].

Direct oral anticoagulants that specifically inhibit thrombin and factor Xa are now available. These agents would not be suitable for all patients, particularly if drug interactions with potential antiviral medication are expected, or in mechanically ventilated patients requiring enteral feeding. Although rivaroxaban and apixaban can be delivered *via* a nasogastric tube to the stomach, they should not be mixed with enteral nutrition [101]. As with LMWH there is an increased risk of bleeding with direct oral anticoagulants but in COVID-19, complications related to bleeding are anecdotally not commonly seen, but a recent study has suggested an increased risk of bleeding in non-critically ill patients receiving heparin [102]. Hence the risk of bleeding complications may be lower for patients at increased risk of death with COVID-19 compared to other causes of severe sepsis/ARDS. Furthermore, idarucizumab and andexanet alfa are now available for the reversal of dabigatran and rivaroxaban/apixaban, respectively, mitigating the fear that the effects of these agents cannot be reversed should bleeding occur.

APC showed beneficial effects in the PROWESS study, particularly in the subgroup of patients with CAP and a high risk of death. Heterogeneity within patient populations has hampered previous studies of APC; any future trials in COVID-19 will need patients to be carefully sub-phenotyped because the benefit in COVID-19 will likely be seen in those at high risk of death with low endogenous levels of APC. Nebulised streptokinase has shown reduced mortality in one study of ARDS patients [79] and fibrinolysis is currently being targeted in COVID-19 with trials of nebulised and systemically administered tPA; these trials are welcomed and have the potential to reduce pulmonary microthrombi and lung injury.

Targeting PAR₁ is another potential attractive approach that could counter the negative effects of both thrombin formation and activation of pro-inflammatory pathways by the coagulation system. Although PAR₁ antagonists are unlikely to impact on VTE they could have beneficial effects in COVID-19 pneumonia through several potential mechanisms: 1) direct anti-platelet effect potentially reducing the incidence of coronary and cerebral artery thrombosis [103]; 2) inhibition of inflammatory signalling pathways downstream of thrombin resulting in attenuated inflammatory responses and inflammatory cell recruitment; 3) protection from the development of post-ARDS pulmonary fibrosis [104]; 4) maintaining integrity of the endothelial alveolar barrier, reducing pulmonary oedema; and 5) indirectly attenuating coagulation activation by reducing inflammation. Several PAR₁ antagonists are in development but only one has been clinically approved, vorapaxar (Zontivity). Vorapaxar is a highly selective small molecule PAR₁ antagonist but its long half-life (20 h) and inhibitory effects on platelets (24–48 h) [105, 106] call for cautious use in critically ill patients, particularly because a reversal agent does not exist. Trials in the secondary prevention of acute coronary syndromes have shown that there is also potential for bleeding, although in these trials patients were on dual anti-platelet medication. PAR₁ antagonism could therefore represent a potential approach to halt progression of the disease in hospitalised patients at risk of critical illness. Future research and carefully designed trials in this area would be welcomed. Table 1 provides a summary of the effects of therapeutics targeting coagulation proteinases in pneumonia, lung injury and sepsis in preclinical and clinical studies.

Conclusions

Preclinical studies have shown beneficial effects of targeting coagulation proteinases in models of pneumonia and lung injury and have added significant knowledge to the understanding of the crosstalk

TABLE 1 Effects of endogenous anticoagulants or drugs targeting coagulation proteases: evidence from preclinical and clinical studies

Pathway	Agent	Animal study outcomes		Clinical study outcomes		Recruiting COVID-19 trials
Tissue factor	TFPI	Coagulation activation	Reduced [42, 107, 108]	Coagulation activation	Reduced [37, 109]	NCT04416048 NCT04394377 NCT04351724
		Inflammation	Reduced [36, 110, 111]	Inflammation	Reduced [37]	
Bacterial clearance	No effect [108, 112]	Mortality	No effect [109]			
	Increased [110]		Reduced [40] [#]			
			No effect [37, 39, 41] ^{¶,†}			
			Increased [37, 39] [§]			
Factor X	Rivaroxaban	Inflammation	Reduced [113]			
	Apixaban ^f	Alveolar leak	Reduced [113]			
Thrombin	Anti-thrombin Dabigatran ^f Bivalirudin ^f	Coagulation activation	Reduced [46]	Mortality	No effect [45, 47]	
					Reduced mortality in subgroup not receiving concomitant heparin [47]	
		Inflammation	Reduced [42, 46]	Risk of bleeding	Increased [47] [§]	
		Alveolar leak	Reduced [42, 46]			
		Bacterial clearance	Increased [46]			
	Heparin	Coagulation activation	Reduced [46]	Mortality	No effect [51–53, 79]	
			No effect [42]			
		Inflammation	No effect [42, 46]			
	Alveolar leak	No effect [42, 46]				
	Bacterial clearance	No effect [42, 46]				
APC	rhAPC	Coagulation activation	Reduced [46, 60]	Coagulation activation	Reduced [63]	
		Inflammation	Reduced [61]	Lung injury score	Reduced [63]	
			No effect [60]			
		Alveolar leak	Reduced [61]	Mortality	Reduced [22]	
				No effect [55, 65–67]		
Fibrinogen	tPA	Fibrosis	Reduced [61]			
		Bacterial clearance	No effect [61]			
	uPA	Coagulation activation	No effect [74]	Mortality	Reduced [79]	
		Inflammation	Reduced [78]			
		Alveolar leak	No effect [74, 76]			
		Reduced [76, 78]				
		No effect [74]				
PAR-1	Vorapaxar RWJ-58269	Fibrinolysis	Increased [74, 78]			
		Coagulation activation	Reduced [114]			
		Inflammation	Reduced [94, 115]			
		Alveolar leak	Reduced [94, 115]			
		Bacterial clearance	No effect [94, 115]			

TFPI: tissue factor pathway inhibitor; APC: activated protein C; rhAPC: recombinant human APC; tPA: tissue-type plasminogen activator; uPA: urokinase-type plasminogen activator; PAR-1: proteinase-activated receptor-1. [#]: trend towards reduced mortality in TFPI-treated group of community-acquired pneumonia patients who had not received concomitant heparin and in whom the pathogen was identified [40]; [¶]: trend towards reduced mortality in TFPI group not receiving concomitant heparin [39]; [†]: trend towards reduced mortality in TFPI group (trial not powered for effect on mortality) [37]; [§]: difference in risk of major bleeding was not significant in those who did not receive concomitant heparin [47]; ^f: no relevant studies were identified using this anticoagulant.

between coagulation and inflammation in the context of pulmonary infection, sepsis and lung injury. Importantly, these preclinical studies have limitations that affect the potential translation of findings to human disease, and previous clinical trials of endogenous anticoagulants in pneumonia and in sepsis have mostly failed to show benefits on mortality. However, in the context of COVID-19, the evidence of a profound hypercoagulable state with pathogenic contributions of both macrothrombi and microthrombi in severe disease suggest there is an improved risk/benefit ratio in targeting specific aspects of the coagulation pathways. The extensive body of research on coagulation and sepsis provides important background for the design of future trials in patients with COVID-19 pneumonia to assess whether targeting coagulation can reduce inflammation as well as VTE and microthrombi, and potentially affect survival. A large armamentarium of potentially beneficial agents exists, and well-designed randomised clinical trials are now needed to investigate the wide range of anticoagulant and anti-fibrinolytic therapies to determine the optimal strategy.

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