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The interplay between inflammation and thrombosis in COVID-19: Mechanisms, therapeutic strategies, and challenges

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ABSTRACT

Coronavirus disease 2019 (COVID-19), caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can cause life-threatening pathology characterized by a dysregulated immune response and coagulopathy. While respiratory failure induced by inflammation is the most common cause of death, micro-and macrovascular thrombosis leading to multiple organ failure are also causes of mortality. Dysregulation of systemic inflammation observed in severe COVID-19 patients is manifested by cytokine release syndrome (CRS) - the aberrant release of high levels of proinflammatory cytokines, such as IL-6, IL-1, TNFα, MP-1, as well as complement. CRS is often accompanied by activation of endothelial cells and platelets, coupled with perturbation of the balance between the pro-and antithrombotic mechanisms, resulting in thrombosis. Inflammation and thrombosis form a vicious circle, contributing to morbidity and mortality. Treatment of hyperinflammation has been shown to decrease thrombosis, while anti-thrombotic treatment also downregulates cytokine release. This review highlights the relationship between COVID-19-mediated systemic inflammation and thrombosis, the molecular pathways involved, the therapies targeting these processes, and the challenges currently encountered.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, first detected in late 2019 has so far resulted in more than 6.0 million deaths (https://covid19.who.int/) globally. It has since been well documented that hyperinflammation and thrombosis are associated with disease severity and mortality [1,2]. As our understanding of COVID-19 pathophysiology and its clinical correlates expanded, research quickly focused on therapies targeting uncontrolled inflammation and thrombosis. Although different treatment strategies have been proposed, optimal care of patients with severe COVID-19 remains uncertain and continues to be developed. In this review, we provide an overview of the relationship between inflammation and thrombosis in COVID-19, and the most recent evidence on the pharmacological management of patients with severe COVID-19, with a focus on inflammation and thrombosis. We also explore the discrepancies between studies and the challenges facing current management strategies.

2. Homeostasis in hemostasis

Hemostasis is the physiologic process that prevents hemorrhage by forming a clot to seal damaged blood vessel walls. It involves complex interactions of the vasculature, platelets, plasma coagulation, fibrinolytic proteins, and cytokine mediators. The interplay between platelet activation and the coagulation cascade has been demonstrated at multiple levels: 1. Exposure of tissue factor (TF), collagen, and von Wlliebrand factor (VWF) in response to vascular injury simultaneously trigger both activation of platelets and the coagulation cascade; 2. Thrombin generated during the coagulation cascade activates platelets via thrombin receptors protease-activated receptor 1 (PAR1) and PAR4; 3. Activated platelets promote the coagulation process by providing negatively charged membranes as a platform for coagulation to occur and releasing various procoagulants through degranulation. These include TF, fibrinogen, prothrombin, and factors V and VIII.

Hemostasis is tightly regulated by a balance of prothrombotic and antithrombotic mechanisms of endothelial cells and blood (Fig. 1). Endothelial cell integrity and function are crucial in preventing abnormal clotting and limiting the clot to the site of injury. Dysregulation of hemostasis results in thrombosis as seen in coronary artery disease, deep vein thrombosis (DVT), pulmonary embolism (PE) and most recently, COVID-19.

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3. Inflammation and thrombosis in COVID-19

The close relationship between inflammation and coagulation has an evolutionary origin. Invasion of pathogens into the bloodstream poses a lethal threat to the host. Effective host defense requires both efficient inflammatory immune and blood clotting responses to prevent pathogen spread. However, dysregulation of any one component in either of these systems can derail the successful balance, resulting in varying degrees of excess inflammation and thrombosis. This is seen in severe SARS-CoV-2 infection, which is characterized by immunothrombotic dysregulation leading to multiple organ failure and death.

SARS-CoV-2 is an enveloped, positive-strand RNA virus. Host cell entry requires viral spike protein binding to angiotensin-converting enzyme 2 (ACE2) expressed on myriad cells including lung, heart, liver, intestine, kidney as well as endothelial cells [3]. Viral entry is facilitated by spike cleavage by the host cell transmembrane serine protease 2 (TMPRSS2). Infected cells produce and release chemokines to recruit and cytokines to activate immune cells. These cells then secrete additional cytokines to help limit the spread of infection [4]. The regulated release of cytokines, particularly type I interferons, early in SARS-CoV-2 infection is an appropriate host response that helps clear the virus [5]. However, in some patients, dysregulated interferon-mediated responses cause overproduction of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, Interleukin (IL)-6, IL-8, and IL-1β, resulting in what has been called cytokine storm or cytokine release syndrome (CRS) [5]. CRS increases the risk of vascular dysfunction, hyperpermeability, thrombosis, and multiorgan failure. At this point, despite continued viral replication, the disease process is driven largely by the host response rather than the virus itself and may be fatal if not resolved (see details below) [6].

Both cytokine storm and thrombosis, the two main complications of COVID-19, are associated with disease severity. Elevation of thromboinflammatory biomarkers including D-dimer, fibrinogen, VWF, C-reactive protein (CRP), ferritin, complement, as well as cytokines IL-6, IL-1β are correlated with increased severity and mortality in COVID-19 [2,6–12]. The interrelationship between disease severity and thrombosis has been evidenced clinically by the observations that when compared with COVID-19 patients without thromboembolism, patients with thromboembolism have higher rates of intensive care unit (ICU) admission, invasive mechanical ventilation treatment, as well as mortality [1]. Additionally, when compared with non-ICU COVID-19 patients, those admitted to the ICU showed a higher frequency of venous thromboembolism (VTE), despite standard-dose or high-dose thromboprophylaxis [13,14]. Finally, elevated levels of inflammatory cytokines at admission points to an increased risk of developing thrombotic events and death over the course of COVID-19 [15]. Thrombosis in patients with COVID-19 affects both arterial and venous circulation, leading to acute coronary syndrome, stroke, DVT, PE, and microvascular thrombosis [16–20]. Autopsies of COVID-19 patients' lungs exhibited severe coagulation abnormalities, immune cell infiltration, and platelet activation [21].

In general, the interplay between thrombosis and the inflammatory response to infection has been characterized as a positive feedback loop in which inflammation promotes thrombosis, which in turn further enhances inflammation. Inflammation shifts the behavior of endothelial cells from antithrombotic (Fig. 1) to prothrombotic and promotes activation of the coagulation cascade (see details below). Thrombin generated through thrombus formation amplifies inflammation by stimulating cytokine release from cells expressing PARs [22]. Additionally, activated platelets also interact with both inflammatory cells and endothelial cells, promoting NETosis and cytokine release (see details below).

Although the pathogenesis of COVID-19-mediated thrombosis has not yet been fully elucidated, it is thought that aberrant TF exposure to blood and TF-mediated thrombin generation as well as an imbalance between pro-and antithrombotic mechanisms are involved during the overwhelming systemic inflammation. These include three key activities: inflammation-mediated upregulation of prothrombotic mediators, the loss of antithrombotic properties of endothelial cells and the blood, and platelet activation.

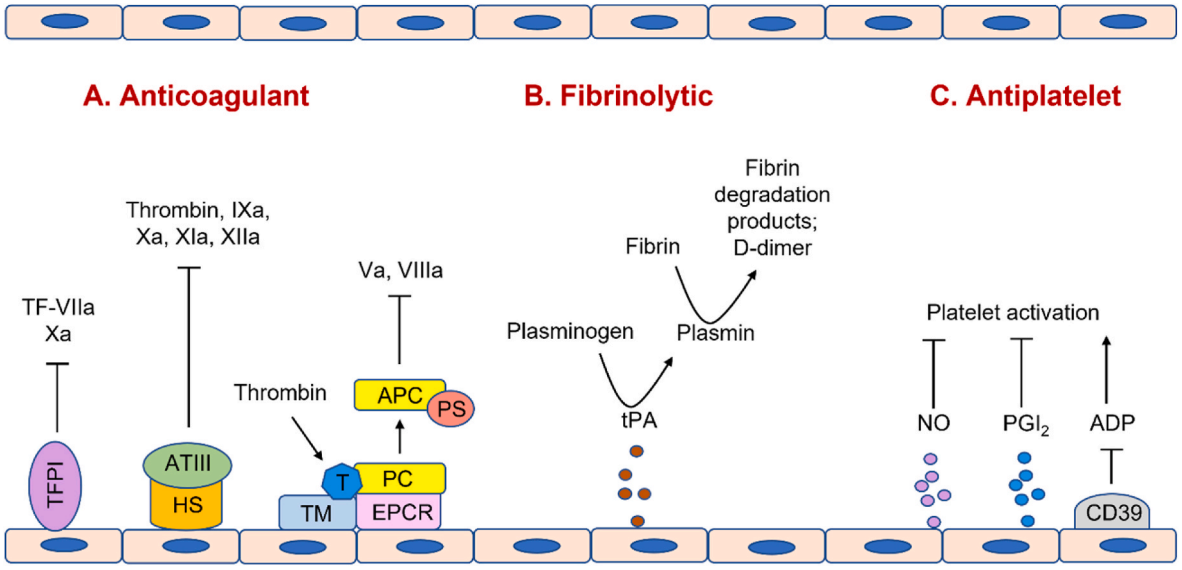

Fig. 1. Physiological antithrombotic properties of endothelial cells and the blood. (A) Anticoagulant properties: TFPI inhibits TF-mediated clotting by binding and neutralizing TF-FVIIa and FXa. Heparan sulphates located on endothelial cell surfaces enhance the capacity of ATIII-mediated inhibition of thrombin, factors IXa, Xa, XIa and XIIa. Thrombin (T)-mediated activation of PC is initiated by thrombin binding to TM on endothelial cell surfaces. EPCR enhances PC activation by binding PC and presenting it to the thrombin-TM complex for activation. Activated PC (APC) then detaches from the EPCR and interacts with PS to inactivate factors Va and VIIIa. (B) Fibrinolytic properties: tPA released from endothelial cells coverts plasminogen into plasmin, which lyses insoluble fibrin into degradation products, including D-dimer. (C) Antiplatelet properties: endothelial production and release of NO and PGI_2, as well as expression of CD39, an ectonucleotidase, inhibit platelet activation.

TFPI, tissue factor pathway inhibitor; HS, heparan sulphate; ATIII, antithrombin III; TM, thrombomodulin; EPCR, endothelial protein C receptor; PC, protein C; APC, activated protein C; PS, cofactor protein S; tPA, tissue plasminogen activator; NO, nitric oxide; PGI2, prostacyclin.

3.1. Role of coagulation in the interplay between inflammation and thrombosis in COVID-19

TF plays a significant role in the initiation of coagulation in both normal hemostasis and inflammation. Under normal conditions, most cells constitutively expressing TF are found in tissues that lack direct contact with blood [23]. Thus, coagulation only occurs when the integrity of the blood vessel wall is disrupted, and TF is exposed to the blood. However, TF expression by endothelial cells and circulating monocytes can be induced under pathological conditions, including inflammation. The crucial role of TF in initiating inflammation-induced coagulation is demonstrated by the observation that blocking TF activity completely inhibits inflammation-mediated thrombin generation in models of experimental endotoxemia or bacteremia [24]. A vast array of inflammatory cytokines, including TNF-α, IL-1, IL-6, lipopolysaccharide (LPS), and CRP have been shown to upregulate TF expression by endothelial cells [25–29] as well as by stimulated monocytes [30,31]. Additionally, cytokines trigger TF producing cells to release TF containing microvesicles into circulation [27]; these have been shown to promote thrombosis both in vitro and in vivo in animal models [32].

The role of SARS-CoV-2 in stimulating immunothrombosis is at least two-fold. First, the virus directly infects vascular endothelial cells via ACE2 and leads to cellular damage and apoptosis [33,34]. It also indirectly induces endothelial dysfunction by stimulating the release of elevated levels of chemokines and proinflammatory cytokines. Endothelial dysfunction in severe COVID-19 patients has been demonstrated by elevated plasma levels of markers of endothelial cell activation and injury, including VWF, soluble P-selectin and soluble TM [35]. Together, this leads to the loss of antithrombotic properties of the normal endothelium (Fig. 1) and shifts the endothelium from antithrombotic to prothrombotic.

A recent study using dual RNA in situ hybridization with SARS-CoV-2 and TF RNA fluorescence probes demonstrates that lung TF expression levels in patients with COVID-19-associated acute respiratory distress syndrome (ARDS) are significantly higher than that in patients with aspiration pneumonia- or bacterial sepsis-associated ARDS. TF expression in the lungs of COVID-19 patients is also associated with fibrin- and platelet factor 4 (PF4)-rich thrombi as compared to non-COVID-19 ARDS lungs and normal controls, suggesting upregulation of TF expression might be implicated in COVID-19 mediated thrombosis [36]. Indeed, COVID-19 patients admitted to the ICU demonstrate elevated levels of monocyte TF expression [7]. Although a full understanding of how SARS-CoV-2 triggers overexpression of TF in the lung continues to be elucidated, mechanistic studies have shown that TF in monocytes is encrypted under normal conditions and this encryption is mediated by sphingomyelin [37]. TF localization changes when human monocyte-derived macrophages are infected with SARS-CoV-2 spike protein pseudovirus (SARS-CoV-2–SP-PV). The virus markedly increases TF procoagulant activity at the cell surface and the release of TF-containing microvesicles. The increased release of TF is mediated by translocation of acid sphingomyelinase (ASMase) to the outer leaflet of the plasma membrane, which leads to the hydrolysis of sphingomyelin [37]. Indeed, COVID-19 patients presenting with elevated levels of circulating microvesicle TF activity are more likely to develop severe or fatal disease [38,39].

As shown in Fig. 1, coagulation is tightly regulated by negative feedback loops and three major physiological anticoagulant pathways: TFPI, PC system and ATIII. During inflammation-mediated activation of coagulation, the function of all three pathways can be impaired. For example, many cytokines that induce procoagulant TF expression also simultaneously reduce anticoagulant activity. An in vivo study using a rabbit model has shown that infusion of IL-1 induces procoagulant TF, while concomitantly blocking the PC anticoagulant pathway in a dose-dependent fashion. TF activity increases >10-fold within 3–5 h after IL-1 infusion, while endothelial cell dependent thrombin-mediated PC activation decreases by 72% and assembly of functional APC-PS complexes on the vessel surface decreases by > 90% [40]. In another study, in vitro incubation of human umbilical vein endothelial cells with the proinflammatory cytokines TNF-α and IL-6 not only increases endothelial TF expression and release of TF containing microvesicles, but also promotes release of a functional truncated soluble form of TF, supporting factor Xa generation in the presence of phospholipids [27]. In addition, proinflammatory cytokines have been shown to suppress endothelial expression of TM [41,42] and EPCR [43,44], two key factors associated with activation of anticoagulant PC (Fig. 1).

Consistently, SARS-CoV-2 infection is also implicated with down-regulation of endogenous anticoagulants including endothelial TM [38], EPCR [21] and ATIII [45,46]. Reduced levels of anticoagulant ATIII in COVID-19 patients have been observed to be correlated with disease severity. Non-survivors amongst hospitalized COVID-19 patients, especially obese individuals, have lower levels of ATIII compared with survivors [45,46]. A soluble form of EPCR (sEPCR) shed from endothelial cells, which exists under normal conditions in plasma, has been found to be elevated in inflammatory conditions [47]. sEPCR binds to both PC and APC with equal affinity. Its binding to APC inhibits the anticoagulant activity of the latter, while its binding to PC precludes PC activation by thrombin/TM complexes [48,49]. Therefore, shedding EPCR from endothelial cells in response to inflammation converts anticoagulant membrane bound EPCR to procoagulant sEPCR. An increase in plasma sEPCR is associated with excess mortality in pneumococcal pneumonia-mediated septic shock [50] as well as disease severity in COVID-19 patients [38,51].

Communication between inflammation and coagulation processes is bidirectional, such that inflammation stimulates coagulation, while activated coagulation factors in turn amplify inflammation directly or indirectly. Although the main function of thrombin is to promote clot formation by activating platelet PAR1 and PAR4, and by converting fibrinogen to insoluble fibrin, thrombin can augment inflammation via PARs, primarily PAR1 on endothelial cells [52]. Thrombin causes endothelial activation and enhanced expression and/or release of many proinflammatory proteins including monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), IL-1, IL-6, and IL-8, all of which enhance inflammation and further injure endothelial cells [52–56]. The TF-FVIIa complex also stimulates PAR signaling, which induces release of inflammatory cytokines and chemokines in vitro and in vivo [57,58]. Activation of coagulation in healthy human subjects by administration of recombinant factor VIIa also elicits a small but significant increase in the levels of IL-6 and IL-8 in plasma. This increase is abolished when the subjects are pretreated with recombinant nematode anticoagulant protein c2 (rNAPc2), an inhibitor of TF [57]. Similarly, in sickle cell disease mouse models, inhibition of TF attenuates inflammation and endothelial cell injury as demonstrated by reduced plasma levels of IL-6, serum amyloid P, and soluble VCAM-1. Additionally, decreased levels of the chemokines MCP-1 and keratinocyte-derived chemokine (KC), as well as myeloperoxidase (MPO) are found in the lungs of sickle cell mice treated with the anti-TF antibody [58].

Taken together, these data paint a picture whereby SARS-CoV-2 infection initiates the production of proinflammatory cytokines, which in turn induce TF expression on mononuclear cells and endothelial cells, thereby inducing thrombin generation and coagulation. Simultaneously, coagulation factors TF-FVIIa and thrombin bind to PARs on platelets and endothelial cells, leading to platelet activation/aggregation that further amplifies inflammatory cytokine release. These processes form a potent positive loop that contribute to uncontrolled inflammation and thrombosis in COVID-19, supporting the rationale for anti-inflammation and anticoagulation therapy in severe COVID-19 cases.

3.2. Role of platelets in the interplay between inflammation and thrombosis in COVID-19

Platelets in COVID-19 patients can be activated through multiple

mechanisms involving the systemic inflammatory reaction, dysfunctional endothelium and endothelial VWF release, thrombin generated from activation of coagulation, and direct effects of SARS-CoV-2 on platelets. Activated platelets secrete granules that contain soluble activators (ADP, epinephrine, and serotonin), adhesive proteins (VWF and fibrinogen) and procoagulants (prothrombin, FV, and FVIII), enhancing thrombosis by recruiting additional nearby activated platelets and promoting fibrin network construction.

Platelet aggregation is a hallmark of COVID-19 associated thrombosis. VWF, released in response to vascular injury, plays a crucial role in mediating adhesion of platelets to extracellular collagen [59]. It is well documented that inflammation induces endothelial dysfunction, which weakens the antithrombotic properties of endothelial cells and increases release of VWF [60]. Consistently, an increase in plasma VWF levels has been observed in COVID-19 patients and is associated with disease severity [10,35]. High concentrations of VWF are also observed in most COVID-19 patient autopsy tissues, including lung, heart, and kidney [21]. In vitro, SARS-CoV-2 can be internalized by platelets in both ACE2-dependent and independent manner. This internalization leads to programmed cell death of platelets and release of prothrombotic intracellular contents, as well as generation of microvesicles [61]. In addition, inflammatory cytokines, such as IL-1β, IL-6 and TNFα, can hyperactivate platelets, leading to pronounced adhesion, morphological changes, and aggregation [62,63]. Upon activation, platelets also release many inflammatory mediators, such as IL-1β, PF4, and express the surface molecules P-selectin and CD40L, facilitating their interactions with endothelial cells and leukocytes. Consistently, significantly higher levels of plasma soluble P-selectin and CD40L, markers of platelet activation, have been observed in critically ill COVID-19 patients compared to non-critically ill subjects [35]. P-selectin cross-links platelets and leukocytes and is a major mediator of platelet-leukocyte aggregation, thereby upregulating leukocyte TF expression, inflammatory cytokine release, and neutrophil extracellular trap (NET) formation (see details below). Platelet CD40L interacts with CD40 on endothelial cells, promoting secretion of chemokines and expression of adhesion molecules by endothelial cells [64,65].

Given the changes in platelet behavior, it is not surprising that COVID-19 is associated with alterations in the platelet transcriptome as evidenced by increased surface P-selectin expression [66]. Circulating platelet-neutrophil, platelet-monocyte, and platelet-T cell aggregates are all significantly higher in COVID-19 patients compared with healthy controls [66,67]. Additionally, platelet-monocyte interaction is strongly associated with upregulation of monocyte TF expression, elevation of coagulopathy markers, as well as disease severity [7]. Platelets from severe COVID-19 patients are able to induce TF expression ex vivo in monocytes from healthy volunteers; this expression can be inhibited by neutralizing antibodies against platelet P-selectin or integrin αIIb/β3 [7].

Ex vivo studies also reveal that platelets isolated from COVID-19 patients themselves are hyperactive, exhibiting increased efficiency in aggregation, adhesion, and spreading [66]. They are also more sensitized to release proinflammatory cytokines and express higher levels of adhesion molecules [68,69] that have been shown to facilitate leukocyte-platelet-endothelial cell interactions, leading to additional inflammatory cell recruitment, cytokine release, NET formation and endothelial cell dysfunction [64,70]. Through these positive loop interactions, platelets have been shown to promote ARDS [71], systemic inflammatory response syndrome [72], and multiorgan failure [73], all of which have been observed/described in severe COVID-19.

3.3. NETs are mediators between thrombosis and inflammation in COVID-19

Neutrophils are critical in innate immunity for their ability to fight pathogen infections. In addition to phagocytosis, neutrophils possess a novel mechanism to defend against pathogens: the release of NETs via a regulated cell death process termed NETosis [74,75]. NETs are networks of extracellular fibers composed of DNA-histone complexes and proteins released by activated neutrophils, including granule derived enzymes MPO and elastase [74]. Various factors stimulate the generation of NETs. These include the presence of pathogen-associated molecular patterns (PAMPs) recognized by pattern recognition receptors (PRR) such as Toll-like receptor (TLR) 4, 7 or 8 [76,77], proinflammatory cytokines [IL-1β, IL-6, C-X-C chemokine 8 (CXCL-8), TNF-α], activated platelets and the complement system via C3, CR1 or C5a [78–80].

NETs represent an important link between inflammation and thrombosis, contributing to a prothrombotic environment through several mechanisms. NETs provide a scaffold and stimulus for platelet adhesion and aggregation [81]. They are also a carrier of prothrombotic molecules, such as coagulation factors, TF containing microvesicles, and VWF [82]. In vivo, TF has been shown to be present in NETs where it promotes fibrin formation [83]. However, the origin of TF within NETs remains debated: it is unclear whether in vivo NET-bound TF is released from neutrophils during NETosis or is entrapped from TF-containing microvesicles released by other cells such as activated endothelial cells, monocytes and platelets [84]. The picture is further clouded by an in vitro study showing that NETs, induced by stimulation of isolated human neutrophils with phorbol myristate acetate or thapsigargin, are found to have no detectable TF activity as measured by the capacity to activate factor X. Indeed, NETs alone have no effect on plasma clotting in a cell-free system. However, when NETs are incubated with endothelial cells, both endothelial TF mRNA expression and activity are elevated in a dose-dependent fashion. NET treated endothelial cells also promote plasma clotting in vitro. This is blocked by TF-neutralizing antibodies, suggesting an important role of NET-induced endothelial TF in the clotting [85]. Furthermore, NET-associated neutrophil elastase degrades TFPI, the main inhibitor of TF-mediated coagulation pathway (Fig. 1) [86].

It is especially noteworthy that NETs can induce macrophages to secrete IL-1β, which in turn enhances NETosis in various diseases, including aortic aneurysms and atherosclerosis [87–89]. NETs also activate endothelial cells and enhance endothelial cell-monocyte interaction by increasing expression of endothelial VCAM-1 and ICAM-1 [85], thereby offering a signaling loop between neutrophils and macrophages. Furthermore, neutrophils carry key components of the complement alternative pathway. Substances released during NETosis can attach to NETs and trigger complement activation, leading to cell injury [90].

Autopsies of lung samples in patients who succumbed to COVID-19 have confirmed the presence of NET-containing microthrombi with neutrophil-platelet infiltration [91]. As compared to controls, patients with COVID-19-associated thrombosis had significantly higher blood levels of NET markers (cell free DNA, MPO-DNA complexes, citrullinated histone H3) and neutrophil activation (calprotectin) [92]. In addition, the levels of NET markers are correlated with disease severity. Incubation of neutrophils, isolated from healthy adults, with plasma from COVID-19 patients induces robust NET formation, whereas plasma from healthy adults does not [91,93]. Mechanistic studies reveal that SARS-CoV-2-mediated NET release from healthy neutrophils depends on neutrophil ACE2 expression and serine protease TMPRSS2 activity, as well as viral replication. Blockade of ACE2 receptors with monoclonal antibody, or inhibition of TMPRSS2 activity by a protease inhibitor abrogates SARS-CoV-2-induced NET release by the neutrophils [94].

Taken together, these data suggest that NETs mediate a positive signal loop between inflammation and thrombosis. If dysregulated, the process that is so effective at clearing pathogens can lead to uncontrolled inflammation and thrombosis as seen in severe COVID-19 patients. As such, NETs may represent potential targets for therapeutic interventions in COVID-19 (see details below).

3.4. Role of complement in the interplay between inflammation and thrombosis in COVID-19

The complement system plays important roles in innate immunity, protecting the host from pathogens. Pathogen invasion triggers one or more complement activation pathways. Each pathway involves a cascade of proteolytic reactions that converge in the activation of the key components C3 and C5 by C3 and C5 convertases, respectively, into bioactive products C3a, C3b, C5a and C5b. C3a and C5a are mediators for general inflammatory reaction. C3b is a major opsonin that decorates targets such as infected cells and immune complexes for phagocytic removal. C5b initiates the formation of the terminal membrane attack complex (MAC; C5b–9), which directly lyses pathogens or damaged cells [95]. Complement activation is tightly regulated and overactivation of the system causes hyperinflammation [96]. Patients with severe COVID-19 have high levels of circulating C5a and soluble C5b-9 that correlate with disease severity [11,12,97]. SARS-CoV-2 itself is able to directly activate the complement system through all three pathways, the lectin, the classical and the alternative pathway [98].

The role of complement in the intercommunication between inflammation and thrombosis has been demonstrated in multiple layers. Thrombin and factors IX, X, and XI promote complement C5 cleavage [99,100], in turn activation of complement promotes coagulation cascade [101,102] and platelet activation [103]. Complement also mediates interaction among endothelial cells, inflammatory cells and platelets. Both C5a and the MAC induce chemokine release and upregulation of adhesion molecules on endothelial cells, which recruits and activates neutrophils and macrophages [104–106]. C3a, C5a and the MAC also stimulate endothelial release of P-selectin and VWF, expression of TF and shedding of TM, which triggers platelet activation and coagulation cascade [106–109]. Moreover, C3a and C5 enhance NETosis [80] that mediates a positive signal loop between inflammation and thrombosis as discussed above.

Given the contribution of complement in inflammation and thrombosis as well as its relationship to disease severity in COVID-19, it is worth exploring whether complement inhibitors are potential therapeutics in treatment of COVID-19.

4. Management of COVID-19 patients with systemic inflammation and thrombosis

Hyperinflammation and thrombosis are frequently associated with severe COVID-19 in which inflammation may be both a cause and consequence of thrombosis. Therefore, anti-inflammatory agents and antithrombotics are critically important in management of immunothrombosis in severe COVID-19 patients. Treatment guidelines are continuously updated based on ongoing advances in understanding of disease pathogenesis and availability of new clinical trial data. Below we discuss current approaches and investigational strategies proposed in the management of severe COVID-19 patients at high risk of developing immunothrombosis. Antithrombotic drugs include anticoagulants (heparins, TF inhibitors, ASMase inhibitors), antiplatelets (aspirin, P2Y12 inhibitors, dipyridamole, VWF inhibitors) and fibrinolytics (tenecteplase). Anti-inflammatory drugs include non-selective glucocorticoids and selective inhibitors of IL-6/IL-6R, IL-1/IL-1R, JAK, NETs and complement. In addition, plasmapheresis/plasma exchange is also discussed as an investigational therapy. The discussion focuses on efficacy of these strategies in terms of clinical outcomes, inflammation, and thrombosis.

4.1. Antithrombotic strategies in management of severe COVID-19

4.1.1. Anticoagulants

4.1.1.1. Heparins. The high rates of thrombosis observed in the COVID-19 patients warrant the use of anticoagulants. Indeed, an early study of 2773 hospitalized COVID-19 patients demonstrated that those treated with systemic anticoagulants had decreased in-hospital mortality and increased median survival (21 days compared to 14 days). The difference was more striking in patients with COVID-19 requiring mechanical ventilation; compared with controls, anticoagulation treatment roughly halved the mortality rate (29.1% vs. 62.7%) and more than doubled median survival (21 days vs. 9 days) [110]. Although there appears to be a consensus about treating all hospitalized patients with some form of anticoagulation, dosing strategies are not yet clear. Most of the clinical trials investigating anticoagulation in patients with COVID-19 (ClinicalTrials.gov) focus on identifying the optimal timing to initiate anticoagulant therapy and the types and optimal doses of anticoagulants in various clinical situations, including hospitalized/ICU-admitted patients, outpatients, as well as discharged patients [111–114]. In addition, some trials investigate the efficacy of combining anticoagulation or/and anti-aggregation with other therapeutics (anti-inflammatory agents, statins).

The common anticoagulants used in COVID-19 are low molecular weight heparins (LMWH) or unfractionated heparin (UFH) for patients with a glomerular filtration rate (GFR) < 30 mL/min. Heparins bind to ATIII and potentiate ATIII-mediated inactivation of thrombin and other proteases including FXa (Fig. 1). Heparins also have several non-anticoagulant properties and can exert anti-inflammatory effects. Indeed, heparins block P-selectin, thereby limiting crosstalk between platelets and neutrophils [115]. Additionally, heparins inhibit neutrophil response and NET formation [116], and reduce the release of IL-1β, IL-6, E-selectin and ICAM-1 [117], which may be beneficial in dampening CRS in COVID-19 patients.

Despite the use of thromboprophylaxis, thrombosis still occurs in some COVID-19 patients and is associated with high mortality [18,118]. In a systematic review of 2928 ICU managed patients treated with anticoagulants (the type, dose, initiation time and duration varied among studies), thrombotic complications have been found to be as high as 34%, with DVT reported in 16.1% and PE in 12.6% of the patients [118]. To accelerate the generation of evidence and maximize the validity of the results, one clinical trial integrated multiple randomized clinical trials including the REMAP-CAP (ClinicalTrials.gov Identifier: NCT02735707), ACTIV-4A (ClinicalTrials.gov Identifier: NCT04505774) and ATTACC (ClinicalTrials.gov Identifier: NCT04372589) to study the effect of therapeutic-dose anticoagulation in hospitalized COVID-19 patients. The results show that initial treatment with therapeutic-dose heparins, compared with prophylactic-dose in non-critically ill COVID-19 patients increased the probability of survival during hospital stay, reduced ICU admission and need for organ support [113]. However, therapeutic-dose heparin treatment conferred no benefit over prophylactic-dose in critically ill patients [112].

Thus, the net effect of anticoagulation on clinical outcomes in patients with COVID-19 may depend on various factors, including the timing of initiation in relation to disease course, the severity of illness in terms of the degree of thrombosis and inflammation, as well as individual thrombotic risk factors. For example, low levels of anticoagulant ATIII in COVID-19 patients has been associated with disease severity [119]. Given that ATIII mediates heparins' anticoagulant effect, severe deficiency of ATIII in COVID-19 can lead to heparin resistance and failure in preventing thrombosis, suggesting that ATIII supplementation therapy with fresh frozen plasma (FFP) in patients with COVID-19-associated hypercoagulopathy may improve thrombosis prophylaxis and thus have an impact on survival [119].

4.1.1.2. TF inhibitors. Upregulation of TF expression on mononuclear and endothelial cells as well as circulating TF are strongly associated with COVID-19-mediated thrombosis. The important role of TF in both inflammation and thrombosis renders it a potential target for therapy. Increasing experimental evidence has suggested that TF inhibition may

have beneficial effects in diseases that prominently feature thrombosis and inflammation. AB201 (from ARCA biopharma) is a small recombinant nematode anticoagulant protein c2 that selectively inhibits TF. Data suggest this investigational therapy has anticoagulant, anti-inflammatory and antiviral activity that could potentially benefit COVID-19 associated thrombosis and inflammation. AB201 has received US Food and Drug Administration (FDA) Fast-Track Designation and a phase IIb double blinded randomized clinical trial (ASPEN-COVID-19, ClinicalTrials.gov Identifier: NCT04655586) is underway, investigating whether this novel TF inhibitor dampens immunothrombosis and improves clinical outcomes in adult hospitalized COVID-19 patients in comparison with standard heparin therapy.

4.1.1.3. Investigational acid sphingomyelinase (ASMase) inhibitors. TF in monocytes is encrypted under normal conditions. Viral infection can markedly increase TF activity at the cell surface and increase release of TF containing microvesicles. This process is mediated by translocation of endosomal/lysosomal ASMase to the outer leaflet of the cell membrane, hydrolyzing sphingomyelin responsible for maintaining TF in the encrypted state [37]. Pretreatment of monocytes with functional inhibitors of ASMase (FIASMAs) desipramine or imipramine or siRNA silencing ASMase reduces SARS-CoV-2-SP-P-induced TF activity on the cell surface and release of TF containing microvesicles [37]. In addition, fluoxetine, another FIASMA, efficiently inhibits the entry and propagation of SARS-CoV-2 in a cell culture model [120]. As FIASMA agents include many small, well-tolerated compounds that are widely used for a broad range of clinical applications (e.g., antidepressant), exploring these FDA-approved pharmaceuticals may help in preventing or treating COVID-19-associated coagulopathy.

4.1.2. Antiplatelet Drugs

Based on the full involvement and hyperactivation of platelets in COVID-19 and incomplete prevention of thrombosis by anticoagulants, it is plausible that the addition of an antiplatelet agent would improve clinical outcomes. Antiplatelet drugs most commonly explored in the management of COVID-19 are aspirin, P2Y12 receptor antagonists and dipyridamole.

4.1.2.1. Aspirin. Aspirin as an irreversible inhibitor of cyclooxygenase (COX), preferentially binds to and inhibits COX-1 at low doses and decreases platelet synthesis of thromboxane A2, a potent platelet activator, thus inhibiting platelet aggregation and thrombus formation [121]. In addition, aspirin's potential benefits in lung inflammatory injury are thought to be related to reduced platelet-neutrophil aggregates in the lungs, reduced inflammation, and increased production of lipoxin (an anti-inflammatory mediator), which restores pulmonary endothelial cell function [122]. These protective effects may therefore be beneficial in a disease such as COVID19.

However, the role for aspirin in preventing COVID-19 thrombosis is mixed. In an early retrospective, observational cohort study of 412 adult patients with COVID-19, aspirin taken within 7 days before or within 24 h upon admission was associated with a significantly lower rate of mechanical ventilation, ICU admission, and in-hospital mortality after controlling for confounding variables [123]. Another retrospective study of 140 covariate-balanced COVID-19 patients also showed that in-hospital use of aspirin compared to no antiplatelet therapy was associated with a significantly lower cumulative incidence of in-hospital mortality [124]. However, in a later larger randomized controlled trial (RECOVERY, ClinicalTrials.gov Identifier: NCT04381936) that enrolled nearly 15,000 hospitalized COVID-19 patients, addition of daily 150 mg aspirin to standard care did not show significant benefit in reducing 28-day mortality or progression to mechanical ventilation. The aspirin group had a small reduction in thrombosis (4.6 versus 5.3%) and a small increase in major bleeding (1.6 versus 1.0%). Thus, this finding does not support the routine use of aspirin as a treatment for hospitalized COVID-19 patients [125]. The ACTIV-4B (ClinicalTrials.gov Identifier: NCT04498273) is a randomized clinical trial that enrolled 657 symptomatic outpatients investigating the effect of aspirin or apixaban (direct oral anti-Xa anticoagulant) in preventing thrombosis and improving clinical outcomes in the outpatient setting. Compared to placebo (n = 164), treatment with aspirin (81 mg orally once daily; n = 164), prophylactic-dose apixaban (2.5 mg orally twice daily; n = 165), or therapeutic-dose apixaban (5 mg orally twice daily; n = 164) for 45 days did not show significant reduction in the rate of a composite clinical outcome. The study was terminated at this point after enrollment of 9% of participants because of an event rate lower than anticipated [126].

4.1.2.2. P2Y12 inhibitors. ADP is a potent platelet activator. In physiological conditions, it is released from platelet dense granules upon stimulation. ADP binds to and stimulates platelet P2Y12 receptors, leading to platelet activation. This is blocked by P2Y12 inhibitors, such as clopidogrel, prasugrel and ticagrelor.

There is a paucity of data regarding the efficacy of addition of P2Y12 inhibitors as adjunct to standard of care of COVID-19 patients. ACTIV-4A (ClinicalTrials.gov Identifier: NCT04505774), an open-label, adaptive randomized controlled clinical trial enrolling 562 non-critically ill patients hospitalized for COVID-19, investigated the efficacy of administration of P2Y12 inhibitors on top of therapeutic anticoagulation therapy. In this study, patients were randomized in a 1:1 ratio to a therapeutic dose of heparin plus a P2Y12 inhibitor (ticagrelor or clopidogrel, n = 293) or a therapeutic dose of heparin only (n = 269) for 14 days or until hospital discharge. The results show that addition of a P2Y12 inhibitor to therapeutic-dose of heparin, compared with heparin alone, did not increase odds of improvement in organ support-free days within 21 days during hospitalization in non-clinically ill hospitalized patients [127]. As part of the ongoing ACTIV-4A trial, patient enrollment continues for evaluating efficacy of P2Y12 inhibitors as an adjunct to prophylactic dose anticoagulation in critically ill COVID-19 patients (ClinicalTrials.gov Identifier: NCT04505774), a population in whom therapeutic dose anticoagulant therapy was not found to be superior to a prophylactic dose of anticoagulant therapy [112].

4.1.2.3. Dipyridamole. The anti-platelet effect of dipyridamole is primarily mediated by inhibition of platelet phosphodiesterases (PDEs), thereby preventing degradation of cAMP and cGMP. Elevated intracellular levels of these molecules mediate inhibition of platelet activation [128–130]. In addition to its antiplatelet effects, dipyridamole has been shown to have anti-inflammatory [131,132] and antiviral activity [133–135]. Therefore, it is of interest to explore the potential beneficial effects of this drug in treatment of COVID-19.

A small cohort study of 31 COVID-19 patients receiving ribavirin, glucocorticoids, and oxygen as standard therapy showed that addition of dipyridamole was associated with markedly improved clinical outcomes and coagulation profiles [135]. There are 5 clinical trials registered with ClinicalTrials.gov investigating the efficacy and safety of the use of dipyridamole in treatment of COVID-19. As of May 2022, three trials were completed, including "Dipyridamole to Prevent Coronavirus Exacerbation of Respiratory Status (DICER) in COVID-19" (NCT04391179), "Evolution of COVID-19 in Anticoagulated or Anti-aggregated Patients (CORONA Study, NCT04518735) and "Aggrenox to Treat Acute COVID-19" (NCT04410328); no interpreted study results are yet published. Two ongoing trials are as follows: 1. "Trial of Open Label Dipyridamole-In Hospitalized Patients With COVID-19" (NCT04424901), a randomized, open-label study evaluating antithrombotic and antiviral effects of adjunct dipyridamole therapy vs. standard care in hospitalized COVID19 Patients; 2. "Brequinar Combined with Dipyridamole in Patients with Mild to Moderate SARS-CoV-2 Infection" (NCT05166876), a randomized, assessor-blind, multicenter, placebo-controlled study assessing the safety and anti-coronavirus efficacy of brequinar (a quinoline carboxylic acid derivative) combined

with dipyridamole in patients with mild to moderate SARS-CoV-2 Infection (ClinicalTrials.gov). More clinical data are needed to draw a conclusion on the efficacy of adjunctive dipyridamole therapy in COVID-19 patients in preventing thrombosis as well as improving virus clearance and clinical outcomes.

4.1.2.4. VWF inhibitors. On the basis of the critical role of VWF in COVID-19 associated thrombo-inflammatory complications, targeting VWF is an attractive potential intervention; to date there are no clinical trials investigating the effects of inhibition of VWF in COVID-19. However, several approaches to reducing VWF levels in acquired thrombotic thombocytopenic purpura (aTTP) may be helpful in the management of thrombosis in SARS-CoV-2 infected patients. aTTP is a condition in which VWF is hyperactive due to deficiency of its specific cleaving protease ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) that downregulates the platelet adhesive properties of VWF by cleaving it into smaller multimers [136]. VWF plays a pivotal role in the initial phase of platelet thrombus formation through the interaction between its A1 domain and platelet GPIb [137]. Therefore, inhibition of VWF A1 domain binding to platelet GPIb may potentially prevent the development of platelet thrombus formation. Caplacizumab, an anti-VWF A1 domain nanobody, shows faster recovery of platelet counts, fewer plasma exchange sessions, and shorter hospital stays in patients with aTTP [138] and is approved for treatment of this condition in Europe and the United States. ARC1779, an aptamer to the VWF A1 domain, was also evaluated in a clinical trial for treatment of aTTP. The trial showed no serious adverse events such as bleeding, even in patients who had severe thrombocytopenia [139]. Recently a novel DNA aptamer, TAGX-0004, targeting VWF A1 domain has been developed. It is currently under preclinical investigations. In comparison with caplacizumab, TAGX-0004 displays equally potent binding to VWF and inhibition against thrombosis formation under different blood flow conditions [140]. In addition to directly targeting VWF, indirectly controlling VWF activity by supplementation of recombinant ADAMTS13 in aTTP was also studied. The SOAR-HI trial (ClinicalTrials.gov Identifier: NCT03922308) is a phase II, double-blind randomized controlled study evaluating the pharmacokinetics, safety, and efficacy of recombinant ADAMTS-13 (SHP655) administered in addition to standard of care treatment in patients with aTTP (n = 28). However, the results of the study are not published yet.

4.1.3. Fibrinolytics

Respiratory failure in COVID-19 is a critical condition that often leads to fatality. Acute respiratory failure is, at least in part, associated with pulmonary micro-and/or macroembolism, creating pulmonary vascular shunt leading to dead-space ventilation. Tenecteplase is a recombinant tPA analogue that is more fibrin selective than natural tPA and is resistant to tPA inhibitors. As a fibrinolytic agent, tenecteplase converts plasminogen into plasmin, which degrades fibrin to its fragments, including D-dimer, and resolves clots. Tenecteplase has been used to treat ischemic stroke, myocardial infarction, and PE. It has been proposed that tenecteplase may benefit PE-associated respiratory failure in COVID-19. So far, two clinical trials are registered with ClinicalTrials.gov investigating the effects of tenecteplase on COVID-19-associated PE and clinical outcomes. Unfortunately, the trial "Low-Dose Tenecteplase in COVID-19 Diagnosed with Pulmonary Embolism" (NCT04558125) was terminated early due to low patient accrual. The ongoing "Tenecteplase in Patients With COVID-19" trial (NCT04505592) focuses on whether tenecteplase with dose escalation in conjunction with heparins can improve clinical outcomes in hospitalized adult COVID-19 patients with ARDS and elevated D-dimer. More clinical trials are needed to evaluate the efficacy of the use of fibrinolytics in COVID-19 ARDS, and most importantly to determine which patients would most benefit from the therapy.

4.2. Anti-inflammatory strategies in management of severe COVID-19

4.2.1. Glucocorticoids

Glucocorticoids bind to cytoplasmic glucocorticoid receptors and translocate to the nucleus where they bind to glucocorticoid response elements in the regulatory regions of target genes. They are potent pan-anti-inflammatory agents and have been widely used in treatment of SARS and MERS to inhibit excessive immune cell activation and cytokine production [141,142]. However, glucocorticoids also suppress the immune response and the host's ability to clear viruses. Based on the results of clinical studies, the administration of glucocorticoids demonstrates no benefit in patients with mild COVID-19 when patients are likely in the process of clearing the virus. By contrast, glucocorticoids are recommended in severe cases in which the disease process is driven by hyperinflammation [143,144]. It should be noted that glucocorticoids have been associated with an increased risk of VTE, especially PE in patients treated for inflammatory diseases [145]. Mechanistic study in healthy volunteers shows that oral administration of prednisolone for 10 days induces a procoagulant state as demonstrated by an increase in speed of thrombin generation and elevated plasma levels of VWF and plasminogen activator inhibitor-1 [146], suggesting that glucocorticoids are procoagulant regardless of a patient's underlying conditions. Therefore, it is not surprising that increased risk of VTE is observed in COVID-19 patients receiving high dose glucocorticoids [147]. Given that many COVID-19 patients treated with glucocorticoids are immobile, and typically have a higher Padua Score and signs of underlying coagulopathy, caution must be taken when treating these patients with high dose glucocorticoids regarding development of thrombosis.

4.2.2. IL-6/IL-6R Inhibitors

IL-6 is a key inflammatory factor in COVID-19 and a crucial mediator in crosstalk between inflammation and thrombosis. Elevated levels of IL-6 are associated with disease severity and mortality in COVID-19 [2,148]. Studies have shown that IL-6 activates endothelial cells and promotes endothelial expression of TF and adhesion molecules, leading to thrombosis. While in turn, FVIIa and thrombin generated in the process of coagulation stimulate endothelial cells to produce and release IL-6 [54,57]. IL-6 binds to either membrane bound IL-6 receptor (mIL-6R) or soluble IL-6 receptor (sIL-6R), forming a complex that acts on cell membrane gp130, thereby regulating levels of cytokines via the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway.

Tocilizumab, a humanized monoclonal antibody (mAb) targeting IL-6R binding to both mIL-6R and sIL-6R, has obtained FDA emergency use authorization to treat critically ill COVID-19 patients who are on systemic corticosteroids and require supplemental oxygen, non-invasive or invasive mechanical ventilation, or extracorporeal membrane oxygenation (ECMO). This approval was based on multiple clinical trials. As summarized in a prospective meta-analysis of 10,930 patients hospitalized for COVID-19 from 27 randomized clinical trials, administration of IL-6R antagonists including both tocilizumab and sarilumab, was associated with lower all-cause mortality 28 days after randomization. The effects were more profound when used in combination with corticosteroids [149].

During treatment, patients with severe COVID-19 who responded well to tocilizumab showed an early reduction in serum levels of inflammatory markers such as CRP, IL-6, IP-10 (also known as C-X-C motif chemokine 10 [CXCL10]), MCP-1, and IFN-γ [150]. Furthermore, levels of platelet/D-dimer ratio, a marker of risk for thrombosis [151] were reduced in the responders [152]. A case series study (n = 70) also demonstrated that treatment with subcutaneous tocilizumab was associated with a rapid and persistent improvement of CRP and coagulation activity as well as respiratory parameters in hospitalized adult patients with COVID-19 pneumonia and hyperinflammation. Reduction in D-dimer and fibrinogen levels were paralleled by improvement in oxygenation, suggesting a role for thrombosis in ARDS. Additionally, the

improvement of coagulation parameters was independent of the use and dose of thromboprophylaxis [153]. All these data suggest an impact of anti-inflammation on thrombotic complications, further confirming the interrelationship between inflammation and thrombosis in COVID-19.

In addition to inhibition of IL-6R, depletion of IL-6 by neutralizing antibody is another way to inhibit IL-6/IL-6R pathway. Siltuximab is a recombinant human-mouse chimeric mAb against IL-6 and is approved by the FDA for use in patients with multicentric Castleman disease. Siltuximab binds directly to IL-6 and like tocilizumab, inhibits both *cis*- and *trans*-IL-6 signaling by preventing IL-6 from binding to mIL-6R and sIL-6R, respectively. There is limited data on the efficacy of siltuximab in treatment of COVID-19. A small cohort of 31 severe COVID-19 patients who received siltuximab showed that it was a well-tolerated alternative to tocilizumab when administered as a first line option in patients with COVID-19 pneumonia with high levels of CRP within the first 10 days from symptom onset [154]. No data have been published in terms of how siltuximab affects thrombotic events or markers in COVID-19. Currently there are 4 ongoing clinical trials registered with ClinicalTrials.gov investigating therapeutic efficacy and safety of siltuximab in treatment of critically ill COVID-19 patients. They are "Efficacy and Safety of Siltuximab vs. Corticosteroids in Hospitalized Patients with COVID-19 Pneumonia" (NCT04329650), "An Observational Study of the Use of siltuximab (SYLVANT) in Patients Diagnosed With COVID-19 Infection Who Have Developed Serious Respiratory Complications" (NCT04322188), "Treatment of COVID-19 Patients with Anti-interleukin Drugs" (NCT04330638), and "Anti-IL6 and Corticosteroid Monotherapy vs Combination in COVID-19" (NCT04486521). No study results are yet published.

4.2.3. Recombinant Soluble gp130 Protein

It is generally believed that IL-6 exerts both pro- and anti-inflammatory effects. The pro-inflammatory effects of IL-6 occur via the *trans*-signaling pathway using sIL-6R and gp130 that is expressed on all cells of the body, regulating synthesis and release of cytokines. On the other hand, the anti-inflammatory and regenerative effects of IL-6 involve the *cis*-signaling pathway via the mIL-6R, present on macrophages, neutrophils, some T lymphocytes, and hepatocytes [155,156]. As tocilizumab and siltuximab are not selective, inhibiting both *cis*- and transactivation signaling of IL-6, this may be one of the mechanisms causing negative side effects such as upper respiratory tract infections [157]. Therefore, it is suggested that recombinant soluble gp130 protein (sgp130) may be an alternative to tocilizumab because it reduces IL-6 pro-inflammatory effects by preventing sIL-6R:IL-6 complex from binding to cell membrane gp130 [156,158]. Clinical trials are needed to evaluate the efficacy and safety of sgp130 in treatment of COVID-19.

4.2.4. IL-1/IL-1R Inhibitors

Like IL-6, IL-1 is another key inflammatory cytokine associated with COVID-19 hyperinflammation. However, clinical trials on efficacy of IL-1/IL-1R inhibition in COVID-19 show variable results. Currently, anakinra, a recombinant IL-1R antagonist, and canakinumab, a mAb targeting IL-1β, are being investigated as potential therapy for COVID-19.

Urokinase-type plasminogen activator (uPA) is a serine protease involved in tissue remodeling and cell migration. Its receptor uPAR is expressed on immune cells (neutrophils, lymphocytes, monocytes/macrophages), endothelial and tumor cells. After cleavage from the cell surface, soluble uPAR (suPAR) is released in the blood and other organic fluids. Elevated levels of blood suPAR has been associated with inflammatory diseases, including systemic inflammatory response syndrome, active tuberculosis disease and human immunodeficiency virus (HIV) infection [159]. In addition, suPAR has been identified as a prognostic biomarker in sepsis [160]. Consistently, blood suPAR is also an early predictor of severe respiratory failure in patients with COVID-19 pneumonia [161]. Using suPAR level as a guide, the SAVE-MORE, a double blinded, randomized controlled phase III trial (ClinicalTrials.gov Identifier: NCT04680949), enrolled 594 hospitalized patients who were receiving dexamethasone and had plasma suPAR levels $\geq$6 ng/mL. These patients were randomized to either anakinra therapy (subcutaneously as 100 mg once daily for 10 days) or placebo. The results showed that patients who received anakinra had lower 28-day mortality and shorter hospital stay than those who received placebo [162]. By contrast, three other clinical trials showed no benefit in targeting IL-1β or IL-1R. These trials include: 1. CORIMUNO-ANA-1 (ClinicalTrials.gov Identifier: NCT04341584), a randomized controlled, open label trial that compared the use of anakinra (via IV infusion as 200 mg twice a day on days 1–3, 100 mg twice on day 4, 100 mg once on day 5) to usual care in 116 hospitalized patients with moderate to severe pneumonia, found anakinra did not improve outcomes [163]; 2. REMAP-CAP (ClinicalTrials.gov Identifier: NCT02735707), an open-label, adaptive platform, randomized controlled trial that evaluated several immunomodulators in patients with COVID-19 who required organ support, found that anakinra given intravenously as 300 mg loading dose, followed by 100 mg every 6 h for 14 days was also ineffective in reducing the combined endpoint of in-hospital mortality and days of organ support (preprint not published as peer reviewed yet); 3. CAN-COVID (ClinicalTrials.gov Identifier: NCT04362813), a randomized, double-blind, placebo-controlled phase III trial that evaluated canakinumab in 454 hospitalized patients with COVID-19 who were hypoxemic but did not require ventilatory support, reported that single intravenous infusion of canakinumab (450 mg for body weight of 40-$<$ 60 kg, 600 mg for 60–80 kg, and 750 mg for $>$ 80 kg; n = 227) did not improve the likelihood of survival without invasive mechanical ventilation [164]. Although both in vivo and in vitro studies have shown that anti-IL-1 antibody significantly inhibited LPS-mediated upregulation of plasma and endothelial TF activity [165,166], the above IL-1 inhibition clinical trials did not evaluate thrombotic events or markers in their studies.

The discrepancy of the outcomes among IL-1/IL-1R inhibitor clinical trials could be attributed to the patient selection, timing of the treatments, doses of the drugs administered, as well as other compounding factors including concomitant treatment with other therapeutics, comorbidity, and genetic variability of the patients. Nevertheless, based on the results from published clinical trials, there is insufficient evidence for the COVID-19 Treatment Guidelines Panel to recommend either for or against the use of anti-IL-1/IL-1R for the treatment of COVID-19.

4.2.5. JAK Inhibitors

JAK family-non receptor tyrosine kinases, including JAK1, JAK2, JAK3 and TYK2, mediate signaling of many cytokines in diverse cell types. JAK inhibitors offer broader anti-inflammation and immunoregulation in comparison with mAbs and recombinant proteins that target a single type of cytokine or cytokine receptor such as IL-6, IL-6R, IL-1, IL-1R, TNF-α and others [167]. JAK inhibitors have been approved to treat many autoimmune diseases, including rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis. Therefore, JAK inhibitors may have a role in combating hyperinflammation in COVID-19. However, several JAK inhibitors have FDA black box warnings for venous and arterial thrombotic events including DVT, PE and ischemic stroke (FDA website). It is unclear whether the presumed prothrombotic risks are dependent on JAK selectivity, drug specificity, dose, treatment duration, or are confounded by indication [168,169]. Therefore, the efficacy and safety of JAK inhibitors in different COVID-19 populations should be carefully evaluated, given that COVID-19 itself is associated with high risk of thrombosis. Of interest is a clinical trial of a new inhaled formulation of pan-JAK inhibitor TD-0903 (nezulcitinib) that confines drug delivery to the lung, thus limiting the systemic side effects. It is a phase II randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT04402866), investigating the efficacy of the inhaled pan-JAK inhibitor in hospitalized COVID-19 patients with acute lung injury. The study was designed with separate data reporting for parts 1 and 2. Part 1 (n = 25), a multiple-ascending-dose trial, identified the dose of 3 mg nezulcitinib once daily for 7 days as the dose for the part

2 trial (n ~ 200) that evaluates the effect of nezulcitinib on clinical outcomes such as survival and respiratory failure-free rate and SaO2/-FiO2 ratio in COVID 19 patients requiring supplemental oxygen. As of May 2022, no interpreted study results are yet published.

4.2.6. Therapeutics Targeting NETs

Given the unique position of NETs in connecting inflammation and thrombosis [170] and their involvement in COVID-19 [91], inhibiting NET formation is an attractive approach to treat COVID-19 patients. The existing drugs against NETs include DNase and inhibitors of neutrophil elastase (NE) and peptidyl arginine deiminase 4 (PAD4), targeting the components of NETs and preventing NET formation and activity [170]. For example, endogenous inhibitors of NET formation, which may be mediated by inhibiting PAD4, have been isolated from umbilical cord plasma and named neonatal NET-inhibitory factor (nNIF) [171]. In an ex vivo study, nNIF blocked excessive NET formation in healthy neutrophils treated with plasma from COVID-19 patients [91]. The NE inhibitor sivelestat was previously approved to treat ARDS in Japan and South Korea, but it did not increase survival in a meta-analysis of clinical trials [172]. Recently, new generations of potent NE inhibitors, including alvelestat, lonodelestat (POL6014), CHF6333 and elafin have entered Phase I testing [170].

The COSTA trial (ClinicalTrials.gov Identifier: NCT04539795) is a small (n = 15) double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of NE inhibitor alvelestat on top of standard of care in patients hospitalized with COVID-19 lung disease. Alvelestat given twice daily for 5 days, with optional extension to 10 days per investigator judgement, was safe and well tolerated. Alvelestat, as an adjunct to standard of care, resulted in a more rapid improvement as measured by the WHO Disease Severity score of ≥2 in the first 5–7 days compared to placebo plus standard of care. In addition, it also reduced IL-6, CRP, as well as D-dimer levels compared to the placebo arm. The changes in these biomarkers support an effect of early intervention with alvelestat on the inflammatory and procoagulant pathways (announced by UAB and Mereo on December 22, 2021).

A recombinant DNase I (dornase alfa), delivered by inhalation, is approved to dissolve NETs in the airways of patients with cystic fibrosis to clear mucus and improve symptoms [173]. A small (n = 30) pilot trial DORNASESARS2 (ClinicalTrials.gov Identifier: NCT04402970) evaluating the effects of dornase alfa in patients with COVID-19 associated ARDS showed that when given 2.5 mg twice daily in the ventilator circuit for 3 days, along with standard of care for ARDS, dornase alfa improved oxygenation and decreased NETs in the bronchoalveolar lavage fluid. However, thrombotic events or markers were not monitored in the study [174]. Although one concern was the potential negative effect of degrading NETs whose function is in part to fight off invading pathogens, there was no observed significant increase in secondary pulmonary infections in the study [174]. These findings warrant more extensive clinical trials to further explore the efficacy and safety of NET inhibitors in treatment of COVID-19-associated ARDS.

4.2.7. Complement inhibitors

Strategies to target complement in COVID-19 have mainly used drugs already in ongoing clinical trials or currently approved for clinical use in known complement-mediated conditions, such as a typical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria.

As reviewed by Afzali and colleagues [98], initially, a few case series of C5-targeting therapies were trialed in a total of 16 patients with severe COVID-19 and showed improved clinical outcomes [175,176]. Then a proof-of-concept small nonrandomized trial enrolling 80 patients with severe COVID-19 showed that addition of eculizumab (n = 35), a mAb against C5, improved both survival and thrombotic markers compared to patients treated with standard of care (n = 45) [177]. Based on the results of these studies, several randomized controlled trials targeting C5 in COVID-19 have been initiated. These include trials investigating safety and efficacy of monoclonal antibodies targeting C5 and preventing its cleavage into C5a and C5b such as eculizumab (ClinicalTrials.gov Identifiers: NCT04288713 and NCT04346797) and ravulizumab (ClinicalTrials.gov Identifiers: NCT04570397 and NCT04369469), and a small peptide C5 antagonist zilucoplan (ZILUCOV, ClinicalTrials.gov Identifier: NCT04382755). Most of these trials are recruiting, not yet recruiting, or have no study result yet. The phase III trial with ravulizumab (NCT04369469) in patients with COVID-19 receiving mechanical ventilation at randomization was terminated based on the interim analysis showing no clinical benefit.

Trials are also studying the impact of blocking either C5a or C5aR. The PANAMO trial (ClinicalTrials.gov Identifier: NCT04333420) is a small (n = 30) open-label, phase II randomized trial investigating safety and efficacy of C5a-targeting antibody vilobelimab in patients with severe COVID-19. Compared to the control arm (n = 15), intravenous administration of vilobelimab (n = 15) had a trend to increase survival and reduce incidence of PE [178]. Because the study was not powered on these endpoints, it has been expanded to a larger multicenter double-blind randomized controlled phase III trial involving 390 patients receiving mechanical ventilation (NCT04333420) and is currently recruiting patients. Similarly, a phase II/III randomized clinical trial (ClinicalTrials.gov Identifier: NCT04449588) involving 368 participants addressing the safety and efficacy of C5a-specific antibody BDB-001 in treatment of severe COVID-19 with ARDS is also in progress. Despite promising results of C5aR blockade in a preclinical model [97], development of the C5aR1-specific mAb avdoralimab (Innate Pharma) in treatment of COVID-19 was discontinued after disappointing results of a phase II randomized clinical trial (FORCE, ClinicalTrials.gov Identifier: NCT04371367) in patients with various degrees of COVID-19 severity.

Targeting the C3 pathway is also an attractive strategy. Use of the C3 inhibitor AMY-101 (Amyndas Pharma) in patients with severe COVID-19 was initially published in two case reports with a total of four patients, all of whom subsequently recovered [179,180]. Furthermore, inhibition of C3 demonstrated a broader anti-inflammatory profile compared to inhibition of C5, due to attenuating both C3a, C5a and C5b-9 generation. Administration of AMY-101 was associated with a more robust decline of neutrophil counts, attenuated NET release and faster serum lactate dehydrogenase (LDH) decline. Additionally, AMY-101 also induced broader downregulation of procoagulant and fibrinolytic responses during complement interception. Levels of both D-dimer and thrombin-TM complexes were markedly reduced within the first seven days of treatment with AMY-101 [179]. Based on these results, a larger phase II study with 144 patients (ClinicalTrials.gov Identifier: NCT04395456) investigating the efficacy and safety of AMY-101 for the management of severe COVID-19 patients with ARDS is planned but is not yet recruiting. The C3 antagonist APL9 (ClinicalTrials.gov Identifier: NCT04402060) was discontinued after no difference in mortality was found in a phase I/II randomized clinical trial in 65 patients with severe COVID-19.

4.3. Plasmapheresis and Therapeutic Plasma Exchange (TPE) in management of severe COVID-19

Given the significant involvement of systemic inflammation and thrombosis in COVID-19 and their association with disease mortality, plasmapheresis has been considered to be a reasonable method to physically remove, and thus reduce the burden of cytokines, aberrant procoagulant agents, as well as virus load [181]. Especially when combined with TPE (e.g., infusion of FFP or convalescent plasma after plasmapheresis), plasmapheresis may improve organ function not only by removing excessive inflammatory cytokines and upregulated procoagulant proteins but also by supplementing the depleted natural anticoagulants observed in COVID-19. Case series studies showed that TPE on top of standard of care improved clinical outcomes and reduced inflammatory and thrombotic markers [182–184]. In addition, a small pilot study (ClinicalTrials.gov Identifier: NCT04374149) of 10 critically ill COVID-19 patients who met criteria for Penn class 3 or 4 CRS

demonstrated that TPE alone immediately reduced cytokine levels and promptly improved oxygenation in both non-ventilated (n = 4) and mechanically ventilated (n = 6) patients [185]. In their study, all patients received 5% human albumin as replacement fluid except for a single patient who received FFP for only two of five plasma exchanges due to coagulopathy. Unlike other studies reported to date, these 10 patients were free from confounding variables such as previous or concomitant use of convalescent plasma, antiviral, glucocorticoids, and anti-IL-6 agents, truly demonstrating the effect of TPE.

Plasma hyperviscosity has been implicated in COVID-19-associated thrombotic events. Among ICU admitted patients, those who had the highest levels of plasma viscosity also had the highest Sequential Organ Failure Assessment (SOFA) scores, a mortality prediction score based on objective measures of six organ systems, and were experiencing acute thrombotic events [186]. Although the exact molecules contributing to the elevated plasma viscosity in COVID-19 are not well known, acute phase proteins are likely candidates. Fibrinogen, a prothrombotic factor that is significantly elevated in many severe COVID-19 patients, could at least partially contribute to the elevated plasma viscosity and increased risk of thrombosis. A case series report of six COVID-19 patients with hyperviscosity has shown that TPE effectively reduced plasma viscosity, SOFA score, as well as levels of fibrinogen, D-dimer, and CRP [187]. Currently, a randomized controlled trial designed to determine the safety and efficacy of TPE in lowering plasma viscosity and improving patient outcomes in COVID-19 is underway (ClinicalTrials.gov Identifier: NCT04441996).

Among the over ten trials registered with ClinicalTrials.gov investigating the effects of TPE on COVID-19, most of the studies focus on clinical outcomes and levels of inflammation (ClinicalTrials.gov). Only one randomized controlled trial entitled “Therapeutic Plasma Exchange as an Adjunctive Strategy to Treat Coagulopathy and Inflammation in Severe COVID-19 (PExCoV, NCT04613986) is designed to investigate the effects of adjunctive TPE therapy on both coagulopathy and systemic inflammation in severe COVID-19. However, as of May 2022, the study was not yet recruiting. Taken together, TPE shows promise; however, randomized clinical trials are needed to draw a clearer conclusion.

5. Challenges

Based on the relationship between inflammation and thrombosis and their involvement in the severe COVID-19, the therapeutic approach to managing COVID-19 associated immunothrombosis should include both anti-inflammatory and antithrombotic agents. However, there are many challenges. Two top challenges are to identify the right patient population and treat them at the right time of the disease course. There is an urgent need to establish criteria for identifying the patient population who might most benefit from certain treatments, likely through a combination of clinical presentations, respiratory parameters, and individual molecular profile of inflammatory or thrombotic markers (e.g., CRP, ferritin, IL-1, IL-6, sECPR, TF, VWF, complement, D-dimer, fibrinogen, suPAR, ATIII, etc). Appropriate biomarkers can also assist clinical decision making in terms of the time to initiate and the duration to maintain a specific therapy. This is extremely important because inflammation is a key part of the immune defense against pathogens, so introducing anti-inflammation at the wrong time may compromise the body’s natural defense to pathogen invasion. It may also partially explain why glucocorticoids only show beneficial effects in critically ill COVID-19 patients, but not in mild to moderate COVID-19 [143,144]. In another example, blockade of IL-6R by tocilizumab only improved clinical outcomes in severe COVID-19 patients whose IL-6 levels were higher than 40 pg/mL, while it worsened the clinical outcomes in those whose IL-6 levels were lower than 40 pg/mL [188]. Notably, COVACTA tocilizumab trial (ClinicalTrials.gov Identifier: NCT04320615) and an ex-US sarilumab trial (ClinicalTrials.gov Identifier: NCT04327388) are two of the trials that did not show beneficial effects of anti-IL-6/IL-6R in severe COVID-19 patients regarding clinical outcomes. Yet, neither of the studies listed elevated serum IL-6 levels within their inclusion criteria [189,190].

Additionally, the biologic heterogeneity in the enrolled patients among trials may also contribute to the variable results. Significance of patient selection is exemplified in anticoagulation therapy whereby the efficacy of heparins is compromised in patients who have ATIII deficiency, as it is ATIII that mediates anticoagulant action of heparins. Furthermore, doses, and dosing regimens as well as concomitant therapeutics and individual comorbidities and risk factors could all contribute to the mixed responses to a specific therapy. For example, therapeutic-dose heparin, compared with prophylactic-dose showed significant improvement of clinical outcomes in non-critically ill COVID-19 patients [113]. However, it conferred no benefit over prophylactic-dose in critically ill patients [112]. To make it more complex, treating hyperinflammation with glucocorticoids and JAK inhibitors may further increase the risk of thrombosis in COVID-19. Therefore, extreme caution must be taken, and antithrombotic therapy must be enforced. TPE is an effective strategy to remove overwhelming levels of inflammatory cytokines and prothrombotic factors. However, it is critical to administer TPE for the correct duration and volume, to monitor the potential interference with other therapeutics due to the removal of drugs from the circulation, and to practice the proper infection prevention and control measures. All these challenges require further extensive international collaboration to run large trials and gather more evidence, with the ultimate goal of clearer management guidelines for the treatment of COVID-19. With more evidence-based treatments, the battle against SARS-CoV-2 infection will lead to reduced morbidity and mortality of COVID-19.

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Abbreviations

ADAMTS13 a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ACE2 angiotensin-converting enzyme 2
ADP adenosine diphosphate
APC activated protein C
ARDS acute respiratory distress syndrome
ASMase acid sphingomyelinase
ATIII antithrombin III
cAMP cyclic adenosine monophosphate
cGMP cyclic guanosine monophosphate
COVID-19 coronavirus disease 2019
COX cyclooxygenase
CRP C-reactive protein
CRS cytokine release syndrome
DVT deep vein thrombosis
ECMO extracorporeal membrane oxygenation
EPCR endothelial protein C receptor
FFP fresh frozen plasma
FIASMAs functional inhibitors of ASMase
GFR glomerular filtration rate
GPIb/IX/V glycoprotein Ib/IX/V
GPVI glycoprotein VI
HIV human immunodeficiency virus
ICAM-1 intercellular adhesion molecule 1

JAK-STAT janus kinase-signal transducer and activator of transcription
LDH lactate dehydrogenase
LMWH low molecular weight heparin
MAC membrane attack complex
MCP-1 monocyte chemoattractant protein-1
NET neutrophil extracellular trap
nNIF neonatal NET-inhibitory factor
NO nitric oxide
PAMPs pathogen-associated molecular patterns
PAR protease-activated receptor
PC protein C
PDE phosphodiesterase
PGI_2 prostacyclin
PF4 platelet factor 4
PS protein S
PE pulmonary embolism
rNAPc2 recombinant nematode anticoagulant protein c2
SARS-CoV-2 severe acute respiratory syndrome coronavirus 2
sEPCR soluble endothelial protein C receptor
suPAR soluble uPA receptor
TF tissue factor
TFPI tissue factor pathway inhibitor
TM thrombomodulin
TMPRSS2 transmembrane serine protease 2
TNF-α tumor necrosis factor-α
tPA tissue plasminogen activator
TPE therapeutic plasma exchange
UFH unfractionated heparin
uPA urokinase-type plasminogen activator
VTE venous thromboembolism
VWF von Willebrand factor

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