World Journal of

Experimental Medicine

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Complement-mediated microvascular injury and thrombosis in the pathogenesis of severe COVID-19: A review

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Abstract

Coronavirus disease 2019 (COVID-19) causes acute microvascular thrombosis in both venous and arterial structures which is highly associated with increased mortality. The mechanisms leading to thromboembolism are still under investigation. Current evidence suggests that excessive complement activation with severe amplification of the inflammatory response (cytokine storm) hastens disease progression and initiates complement-dependent cytotoxic tissue damage with resultant prothrombotic complications. The concept of thromboinflammation, involving overt inflammation and activation of the coagulation cascade causing thrombotic microangiopathy and end-organ damage, has emerged as one of the core components of COVID-19 pathogenesis. The complement system is a major mediator of the innate immune response and inflammation and thus an appealing treatment target. In this review, we discuss the role of complement in the development of thrombotic microangiopathy and summarize the current data on complement inhibitors as COVID-19 therapeutics.

Core Tip: Current evidence supports the role of excessive complement activation with subsequent illness progression and development of a complement-dependent cytotoxic tissue damage with detrimental effects in coronavirus disease 2019 (COVID-19) patients, including thromboembolic complications. Based on its role in the development of the cytokine storm and thrombogenesis in COVID-19, the complement system is an appealing treatment target with promising results from preliminary reports. Whether inhibition of upstream (C3, C1) or terminal (C5, C5a, or C5aR) components is of equal importance remains to be elucidated, however, preliminary results from several ongoing clinical trials show benefit in terms of 28-d mortality and pulmonary embolism.

INTRODUCTION

Coronaviruses are a large family of enveloped viruses that can cause serious respiratory infections, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19) [from SARS coronavirus 2 (SARS-CoV-2)]. Although COVID-19 symptoms are often mild, up to 20%-25% of hospitalized patients require intensive care[1,2]. A substantial proportion of patients develop respiratory complications such as pneumonia and acute respiratory distress syndrome (ARDS) as part of a dysregulated systemic inflammatory response, in addition to acute renal, cardiac, and hepatic injury and disseminated intravascular coagulation[3]. Disease severity and mortality appear to be associated with patient age and comorbidities, suggesting a dynamic relationship between viral replication and host immune response. Patients with high levels of pro-inflammatory cytokines and chemokines show a greater degree of pulmonary inflammation, a phenomenon that has also been observed with SARS and MERS[4]. Although the molecular mechanisms of viral pathogenicity are not fully understood, immune-mediated damage is a major contributor to SARS-CoV-2-associated morbidity and mortality[5]. Rapid cardiorespiratory failure and multiorgan injury, common features of severe SARS-CoV-2 infection, can be partly explained by an aberrant immune response[6].

The complement system is an important part of the innate immune system and participates in the perivascular and intravascular clearance of pathogens, as well as in coagulation and fibrinolysis. In severe cases, SARS-CoV-2 induces a dysregulated immune response that becomes detrimental to the host, described as 'cytokine storm' or 'cytokine release syndrome'[7]. During cytokine storm, serum levels of complement components 3 and 4 (C3 and C4) and other components of the classical complement pathway as measured by the CH50 assay are decreased due to increased complement factor consumption[8]. Post-mortem cadaveric analysis of patients with severe SARS-CoV-2 infection has demonstrated thrombotic microangiopathy (TMA) implicating the activation of the complement cascade[9]. These observations, coupled with results of proteomic studies, highlight the role of complement activation in the pathogenesis of SARS-CoV-2[10]. In this review, we summarize the current evidence of complement involvement in microvascular injury and thrombosis in SARS-CoV-2 infection, as well as current data on complement inhibitors in the treatment of severe COVID-19.

THE ROLE OF THE COMPLEMENT SYSTEM IN VIRAL INFECTIONS

The complement system is an integral part of the innate immune system consisting of over 30 proteins. There are 3 distinct pathways of complement activation: the classical complement pathway, the alternative complement pathway, and the lectin pathway (Figure 1). The complement cascade mediates several immunoprotective and anti-inflammatory functions, enables clearance of viral pathogens and infected cells *via* opsonization, results in the formation of the C5b-9 membrane attack complex (MAC) on infected cells, targets intracellular viral components for proteasomal degradation, promotes
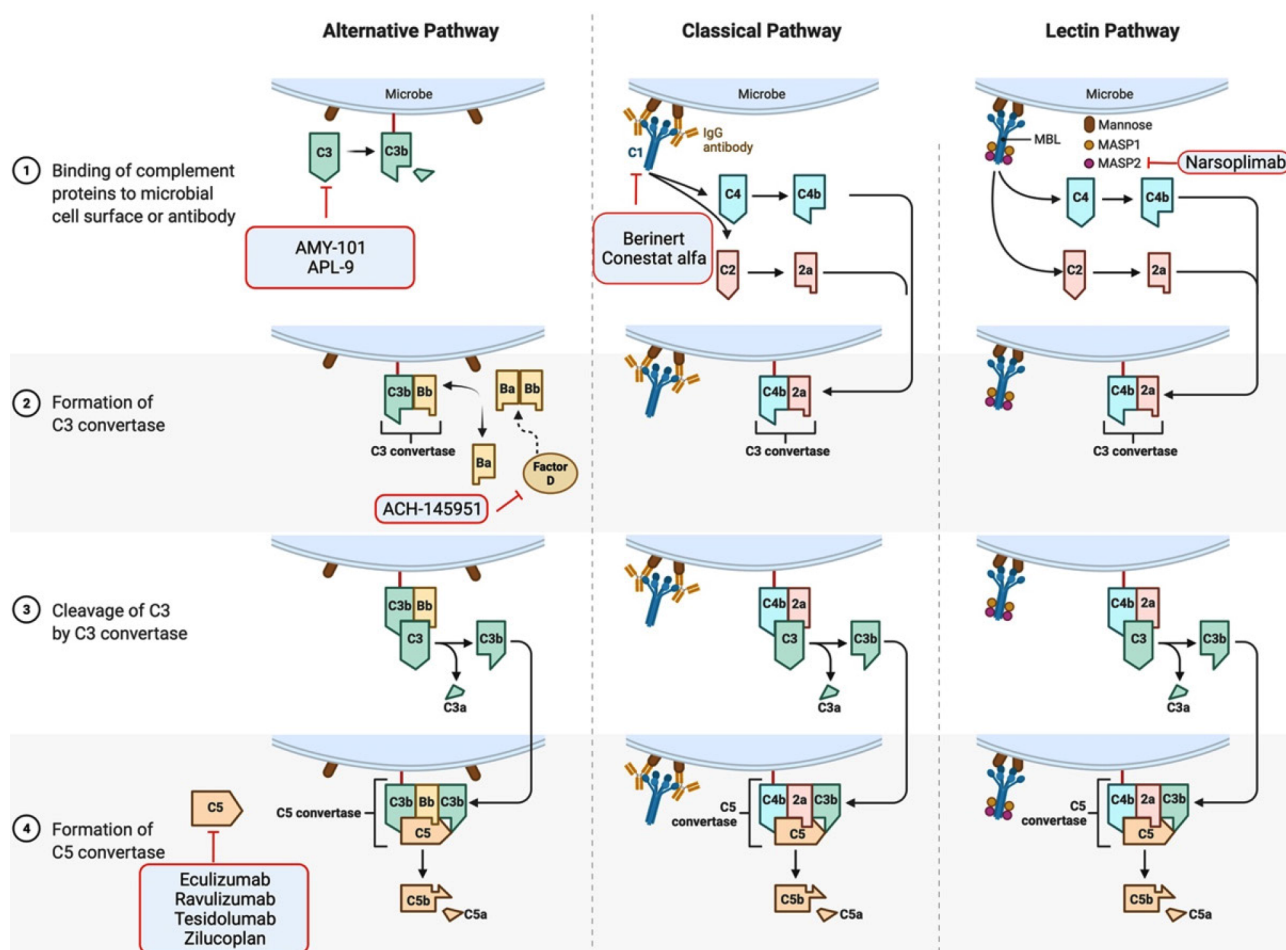
Figure 1 The 3 distinct pathways of complement activation (the classical complement pathway, the alternative complement pathway, and the lectin pathway) and complement inhibitors with potential use in reducing coronavirus disease 2019 related side effects. Created with BioRender.com

chemotaxis, and enhances the adaptive immune response[11]. In addition, the complement system promotes the survival of germinal center B cells and enhances the production of antigen-specific antibodies[12,13]. Further, activation of the complement system leads to the production of anaphylatoxins, such as C3a and C5a, which triggers endothelial and mast cell degranulation, enhances phagocytic activity of neutrophils and monocytes, and elicits a local inflammatory response.

Complement pathway activation plays an important role in the development of acute lung injury induced by highly pathogenic viruses, and accordingly, inhibition of complement components has been associated with protective effects[14]. High levels of C5a have been found in the upper respiratory tract and in serum samples in patients infected with the H1N1 virus and have been moderately associated with disease severity[15,16].

In preclinical studies with rodents, inhibition of C5a and upstream factors, such as C3 and C3a, reduced lung injury caused by coronavirus (SARS-CoV) and non-coronavirus (avian influenza H5N1 virus) infections[17,18]. Interestingly, there was no change in viral titers, suggesting that complement inhibition may prevent lung damage independent of viral load[17]. Further, Jiang *et al*[19] showed that MERS-CoV infection in transgenic human dipeptidyl peptidase 4 (*hDPP4*) mice was associated with elevated cytokine release and excessive complement activation, resulting in increased concentrations of C5 cleavage products in sera and lungs, while competitive antibody-mediated inhibition of the C5a receptor (C5aR) decreased viral replication and mitigated alveolar damage by limiting alveolar macrophage infiltration and interferon (IFN)-gamma receptor expression. Gralinski *et al*[17] showed significantly milder airway inflammation, decreased inflammatory cell infiltration, and lower cytokine levels in both lungs and serum of transgenic C3-deficient mice infected with SARS-CoV compared to wild-type control mice. Similar findings were observed in a primate model of influenza H7N9 virus, where inhibition of C5aR significantly decreased cytokine levels and neutrophil infiltration of the lungs [20].

COMPLEMENT ACTIVATION IN SEVERE COVID-19

Clinical studies of patients with COVID-19 have supported the theory that excessive complement activation and complement-dependent cytotoxic tissue damage drive disease progression[21]. Peffault de Latour *et al*[22] showed that the level of circulating MAC (sC5b-9) was increased in 64% of patients and plasma levels of sC5b9 were significantly higher in infected patients compared to healthy donors. Serum C5 may be associated with COVID-19 severity; those with critical disease have significantly elevated sC5b9 compared to those with mild or moderate symptoms[21,22]. Yu *et al*[23] demonstrated that serum from patients with severe COVID-19 promotes complement-mediated cell death by increasing MAC deposition on the cell surface. A positive modified Ham test (complement-mediated cell-death assay) was detected in 41.2% of intubated patients compared to 6.3% of patients requiring minimal respiratory support. Similarly, Carvelli *et al*[24] reported increased plasma C5a levels associated with disease severity and ARDS. Lastly, Holter *et al*[25] showed that sC5b9 and C4d were significantly higher in patients with respiratory failure and systemic inflammation.

The activation of the complement system results in consumption of C3 and C4 and relevant changes have been investigated as markers of disease severity, intensive care unit (ICU) admission, thromboembolism, and mortality[26]. Both C3 and C4 Levels were significantly lower in severe COVID-19 or deceased patients in a meta-analysis of 19 studies including 3764 patients[27]. Serum levels of C3 were reduced in the majority of a small cohort of healthcare workers with COVID-19, suggesting activation of the complement cascade and C3 consumption[28], while case series demonstrated that lower serum C3 on hospital admission or its progressive decline during hospitalization were associated with up to a 4-fold higher risk of disease progression[29,30]. Confirming these findings, Sinkovits *et al*[31] revealed an association between an increased C3a/C3 ratio and need for intubation/mechanical ventilation and in-hospital mortality, while Zhao *et al*[32] identified decreased C3 and C4 Levels in a cohort of 125 non-survivors hospitalized during the early stages of the pandemic in Wuhan. In contrast to the aforementioned findings, adjusted analysis in a cohort of 100 ICU patients including 81 patients with acute kidney injury demonstrated no association between kidney injury and the level of C3[33].

Dynamic changes of complement levels have been reported in patients with COVID-19. Alosaimi *et al* [34] reported higher C3a, C5a, and factor P (properdin) levels in severe COVID-19 that were also higher in critical COVID-19 non-survivors. Further, the levels were increased during the early stage and gradually decreased during hospital course. Continuous sampling in hemodialysis patients with severe COVID-19 identified that C5a levels were elevated prior to clinical deterioration. C3a levels remained elevated during the severe phase, whereas C5a levels started decreasing on day 7[35]. Interestingly, erythrocytes have been proposed as a diagnostic marker of disease progression based on the expression of complement receptors and complement binding. COVID-19 patients admitted to the ICU had an increased percentage of RBCs coated with C3b/iC3b/C3dg and C4d during the first 72 h of admission and the percentage increased further by day 7 in the study by Lam *et al*[36].

Complement component profiles were investigated by Defendi *et al*[37], who performed an extensive analysis of the functional activities and antigenic levels of individual complement components [C1q, C4, C3, C5, Factor B, and mannose-binding lectin (MBL)] and evaluated their association with clinical outcomes, including rate of ICU admission, corticosteroid treatment, oxygen requirement, and mortality. Two distinct profiles emerged: patients with greater disease severity and mortality exhibited activation of the lectin and alternative pathways and low levels of MBL, C4, C3, Factor B, and C5, while patients with more moderate disease showed inflammatory markers compatible with classical pathway activation.

Genetic polymorphisms of C3 have been identified and associated with COVID-19 susceptibility and mortality[38]. Gavriilaki *et al* used targeted next-generation sequencing and identified C3 variants as independent predictors of disease severity, ICU admission, and/or mortality, strengthening the hypothesis of genetic susceptibility in severe COVID-19[39,40]. Other genetic polymorphisms associated with severe disease include the mannose binding lectin gene 2 (rs1800450)[41,42] and the chromosome 3 rs11385942 G>GA variant that has been associated with complement overactivation (formation of C5a and MAC)[43].

Post-mortem histopathological studies of patients with severe COVID-19 revealed endothelial deposition of complement activation products in the lungs and skin, including C5b9, C3d, C4d, and the mannan-binding lectin serine protease 2 (MASP-2), an important mediator of the lectin pathway activation[9,44]. Similarly, Kim *et al*[45] identified immune complexes and MAC deposition in airways and vasculature of lung biopsies, enhanced viral antigen-specific responses in lung-derived myeloid cells, and significant increases in concentrations of C3a and C5a in critical COVID-19 patients. In a retrospective study of 74 patients with COVID-19, SARS-CoV-2 membrane and spike proteins and MASP-2 were also detected and co-localized in small bowel vessels of those patients with microvascular injury, supporting the role of thromboinflammation and complement activation[46]. Interestingly, binding of the SARS-CoV-2 spike protein S1 and S2 subunits to heparan sulfate on cell surfaces and binding of the S and N proteins to lectin pathway molecules cause excessive activation of the alternative and lectin pathways, respectively, resulting in end-organ damage[47,48]. In contrast to the lung and small bowel findings, Santana *et al*[49] reported a low rate of C4d deposition (22%) in liver histopathologic specimens of 27 deceased patients, suggesting that hepatocellular injury is a result of systemic

rather than intrahepatic thrombotic events.

THE ROLE OF COMPLEMENT IN COVID-19 INDUCED THROMBOTIC MICROANGIO-PATHY

Coagulopathy resulting in a high frequency of thrombotic complications, including venous thromboembolism (VTE) such as deep vein thrombosis and pulmonary embolism, and arterial thromboembolism such as myocardial infarction and ischemic stroke, is common in critically ill COVID-19 patients and is among the leading causes of death[50,51]. The incidence of VTE has been estimated at 5.5% to 14.1% or more – an over two-fold higher risk compared to historical matched cohorts[52-54]. Microvascular thrombosis has been associated with progression to ARDS[55], while autopsy studies have identified VTE or *in situ* pulmonary arterial thrombosis in at least 60% of patients with COVID-19, suggesting thrombosis as a major cause of mortality[56,57].

The causal mechanisms of the COVID-19 coagulopathy are diverse and include dysregulated inflammation (cytokine storm) with subsequent activation of the coagulation cascade and platelets[50,58], virus induced endothelial changes[59-61], or patient comorbidities and limited mobility related to prolonged hospitalization[62]. Increased plasma levels of D-dimer, a marker of coagulation cascade activation, especially greater than 4 times the upper limit of normal, predict a more than two-fold increased risk of VTE or mortality[2,60], while thrombocytopenia and prothrombin time prolongation have also been observed[63].

In the context of thromboinflammation, the complement pathways are capable of activating the coagulation cascade through the induction of tissue factor expression[64,65]. Furthermore, serine proteases of the lectin pathway can cleave prothrombin to form activated thrombin, and MBL has been shown to be significantly increased in critically ill COVID-19 patients with symptomatic thromboembolism[66,67]. Complement system inhibitors, such as C1-esterase inhibitors, can additionally inhibit the coagulation cascade[68].

Current histopathologic data suggest TMA – manifesting as thrombocytopenia, microangiopathic hemolytic anemia, and organ damage – as a potential cause of severe COVID-19. TMA has been widely reported in postmortem studies, particularly as pulmonary capillary stasis and presence of microthrombi in the lungs, along with erythrocyte aggregation, endothelial injury, and fibrin thrombi in kidneys, despite anticoagulation[69,70].

Diffuse alveolar damage and complementmediated endothelial injury of septal microvasculature and microthrombi have been observed in critically ill patients with increased serum D-dimer levels and fibrinogendegradation products, further strengthening the concept of immunemediated pulmonary vascular injury and thrombosis in COVID-19[71]. Lung histopathologic data have also shown that severe COVID-19 is characterized by innate-immunity cell-mediated inflammatory endothelial damage manifesting as an obliterating endarteritis, associated with accumulation of C5aR1+ lung macrophages around the arteries and within thrombi[24,72]. This finding supports the notion that C5a production attracts and activates myeloid cells in the lungs, causing excessive inflammation and endothelial damage[24].

Complement-mediated renal TMA has been investigated in both adults and children with COVID-19, with evidence showing a constitutional complement dysregulation and intrarenal complement activation. These findings have been associated with genetic alterations of the alternative complement pathway and suggest SARS-CoV-2 as an emerging infectious trigger for atypical hemolytic uremic syndrome (aHUS), in accordance with previous cases precipitated by influenza strains[73]. COVID-19-associated renal TMA is characterized by increased deposition of complement components (C1q, C3, C5b9) and total immunoglobulin[74] and unrestrained formation of C5b9[75], which has also been observed in children with COVID-19 independent of disease severity and in the presence of clinical and diagnostic criteria of TMA[76]. Further confirming these findings, Cugno *et al*[77] identified an association between high levels of C5b9 levels and von-Willebrand factor and a positive association with disease severity, suggesting that complement activation and endothelial injury are major determinants of the clinical course of COVID-19 and potential treatment targets.

Cutaneous histopathologic data, derived from chilblain-like lesions, also known as "COVID toes" – inflammatory erythematous papules involving fingers and toes – are characterized by a significant transcriptomic activation of systemic immune response (type I IFN, IgA ANCA), complement activation (upregulation of C1q, C1s and C1 inhibitor, C2, properdin, and downregulation of MAC components C5 and C6), angiogenesis factors (VEGF-A, VEGFR-2 and c-Kit), and endothelial dysfunction (angiopoietin-1, angiopoietin-2 and VEGF-A)[78]. Skin findings may be associated with antiphospholipid antibodies as supported by an analysis of skin samples in a patient with severe COVID-19 with complement-induced vascular injury and severe thrombosis[79] and deposition of C5b9, MASP2, and C4d as shown by skin biopsies in three patients with treatment-resistant COVID-19[80].

Transcriptomic and proteomic analyses have provided important insights in the interaction between inflammation and coagulation pathways in COVID-19. Transcriptomic profiling of leukocytes from intensive care patients revealed the upregulated expression of genes involved in inflammation,

coagulation, and platelet function, concordant with the activation of complement pathways, including *SERPINE1* (plasminogen activator inhibitor-1; PAI-1), von Willebrand factor, and Granzyme B, factors involved in the Toll-like receptor-mediated cascades, and tumor necrosis factor/interleukin 6 (IL-6) signaling[81]. In order to further investigate the proteomic signature and identify biomarkers of disease severity in COVID-19, Barberis *et al*[82] conducted a proteomic profile characterization of plasma-derived exosomes from COVID-19 patients and healthy controls. They reported a specific proteomic signature of strongly regulated proteins in both critically and non-critically ill patients, compared to healthy subjects, including proteins involved in the acute phase response (C-reactive protein [CRP], serum amyloid A, and ferritin), immune-response (C1R, C4A/C4B, MBL2 and SERPING1), and coagulation (proteins of the intrinsic and extrinsic coagulation cascade, Kininogen-1), and reported that the C1r complement subcomponent is highly associated with disease severity, with an AUC of 0.93 (sensitivity: 89%; specificity: 82%). Consistent with the above findings, Freda *et al*[83] observed significant increases in thrombotic and inflammatory marker expression (thrombomodulin, PECAM-1) in human endothelial cells exposed to SARS-CoV-2 structural proteins. Kaiser *et al*[84] analyzed the proteome of neutrophils in severe COVID-19 and reported a unique proteomic signature of increased IL-8 secretion associated with increased D-dimer and neutrophil extracellular trap (NET) production, elevated complement factors (C1R, C1S, C5, C6, C7, C8 and C9), and fibrinogen binding, further uncovering a procoagulant role of inflammation and complement pathways. Lastly, NETs have been implicated in cytokine storm, and inhibition of C3aR and C5aR has been shown to attenuate thromboinflammation driven by NETs[75,85].

COMPLEMENT INHIBITION AS AN EFFECTIVE TARGET WITH THERAPEUTIC IMPLICATIONS

The complement system has garnered interest as a therapeutic target in the treatment of COVID-19. Several clinical trials investigating C1 esterase, C3, C5, C5a, or C5aR inhibition (Table 1) show reduced incidence of 28-d mortality and pulmonary embolism[86].

Most available evidence from case reports, small case series, and ongoing studies has focused on inhibition of C5, C5a, or C5aR. Eculizumab and ravulizumab are humanized monoclonal antibodies, currently used for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and aHUS, that bind to terminal complement component C5 with high affinity, preventing the subsequent formation of C5b9. C5 inhibition can attenuate hyperinflammatory lung damage caused by SARS-CoV-2 in PNH patients with active COVID-19 infection[87]. Those without underlying PNH or aHUS may also potentially derive benefit. In a case series of four patients with severe pneumonia or ARDS, patients received up to 4 infusions of eculizumab and showed a marked improvement in respiratory status and need for non-invasive ventilation within 48 h of the first dose and recovered completely[88]. Similarly, in eight patients with severe or critical COVID-19, six patients showed improved oxygenation after receiving a first dose of eculizumab and were ultimately discharged, while two patients died from septic shock and massive pulmonary embolism, respectively[22]. These findings were further reinforced by Zelek *et al*[89] who reported that Tesidolumab (LFG316), a C5-blocking monoclonal antibody, can rapidly decrease the hyperinflammatory response in 4 out of 5 critical patients with high levels of MAC not responding to standard treatment. Interestingly, in accordance with *in vivo* findings, *in vitro* C5aR inhibition in human airway epithelial cells results in epithelial integrity and promotes anti-inflammatory effects[90].

In patients with established TMA or PNH and concomitant COVID-19, the disease course was milder in those receiving eculizumab or ravulizumab[91,92]. In one of the largest studies (*n* = 80) of complement-targeted therapy in COVID-19, 35 ICU patients treated with eculizumab showed an improved 15-d survival of 82.9% (95%CI: 70.4%-95.3%) compared to 62.2% (95%CI: 48.1%-76.4%) without eculizumab, and improved 28-d survival of 80.0% (95%CI: 66.8%-93.3%) with eculizumab *vs* 51.1% (95%CI: 36.5%-65.7%) without eculizumab accompanied by reduction in key biomarkers (IL-6, IL-17, IFN a2 and C5b9)[93]. However, eculizumab administered in a regular schedule in the treatment of PNH was inadequate in the prevention of ARDS, raising questions regarding the optimal dose and administration in patients with severe COVID-19[94]. A combination of eculizumab with other immunomodulatory agents, such as ruxolitinib, a Janus Associated Kinase inhibitor, may result in improved outcomes and supports the hypothesis that the ideal treatment regimen may be multifaceted [95].

C3 inhibition is also under investigation as a potential therapeutic strategy. Genetic variants of the C3 protein can independently predict risk of developing severe COVID-19, need for ICU-level care, and mortality; this may provide a theoretical foundation for the early use of complement inhibitors[39]. The compstatin-based C3 inhibitor AMY-101 was safely and successfully used in a patient with SARS–CoV-2 associated pneumonia[96]. Further data from an exploratory study by Mastellos *et al*[97] in severe COVID-19 patients treated with eculizumab (*n* = 10) or AMY-101 (*n* = 3), showed attenuation of the hyperinflammatory response, especially with AMY-101. Both agents resulted in a significant decrease in inflammatory markers such as CRP and IL-6 and improved lung function. AMY-101 attenuated C3a and C5b9 levels, decreased fibrinogen consumption, neutrophil counts and NET formation, and enhanced

Table 1 Randomized clinical trials investigating complement inhibitors in the treatment of coronavirus disease 2019

NCT number	Drug	Mechanism of action	Status	Sponsor
NCT04395456	AMY-101	C3 inhibitor	Not yet recruiting	Amyndas Pharmaceuticals S.A.
NCT04402060	APL-9	C3 inhibitor	Completed	Apellis Pharmaceuticals, Inc.
NCT04346797	Eculizumab	C5 inhibitor	Recruiting	Assistance Publique- Hôpitaux de Paris
NCT04355494	Eculizumab	C5 inhibitor	Expanded access no longer available	Alexion Pharmaceuticals
NCT04288713	Eculizumab	C5 inhibitor	Expanded access available	Hudson Medical
NCT04351503	Eculizumab	C5 inhibitor	Recruiting	University Hospital, Basel, Switzerland
NCT04369469	Ravulizumab	C5 inhibitor	Terminated (Met futility bar at interim analysis)	Alexion Pharmaceuticals
NCT04382755	Zilucoplan (RA101495)	C5 inhibitor	Completed	University Hospital, Ghent
NCT04371367	Avdoralimab	Anti-C5aR	Completed	Assistance Publique Hopitaux De; Marseille &Innate Pharma
NCT04414631	Conestat alfa	C1 esterase inhibitors	Terminated	University Hospital, Basel, Switzerland & Pharming Technologies B.V.
NCT04530136	Ruconest	C1 esterase inhibitors	Recruiting	Pharming Technologies B.V.
NCT04333420	Vilobelimab (IFX-1)	C5a	Recruiting	InflaRx GmbH
NCT04570397	Ravulizumab	C5 inhibitor	Recruiting	Brigham and Women's Hospital
NCT04390464	Ravulizumab	C5 inhibitor	Recruiting	Cambridge University Hospitals NHS; Foundation Trust; Frances Hall

lymphocyte recovery.

The classical pathway has been targeted at the level of C1 esterase with inhibitors, such as Conestat alfa and Berinert, that have been previously used in patients with hereditary angioedema[98,99]. Berinert has similar anti-complement effects as heparin, which has demonstrated efficacy in COVID-19 treatment[100-102]. An exploratory study by Urwyler *et al*[103], which investigated Conestat alfa in 5 patients with severe COVID-19, showed improved clinical outcomes such as defervescence and recovery, and improved inflammatory markers levels including CRP, C4d and C5a. Common side effects for Conestat alfa and Berinert include nausea and vomiting alongside with other gastrointestinal symptoms and coinfections[104,105].

SARS-CoV-2 spike protein subunits 1 and 2 can directly activate the alternative pathway through interaction with heparan sulfate on host cell surfaces. This offers another potential therapeutic target as it could be prevented by small molecule inhibitors of factor D (ACH145951)[47]. These molecules bind factor D with high affinity and limit its proteolytic activity against proconvertase (Factor B in complex with C3b). Factor D deficiency is associated with increased risk for recurrent infections with encapsulated organisms comparable to other terminal complement deficiency syndromes[106].

The lectin pathway has been targeted with narsoplimab, an anti-MASP-2 monoclonal antibody, in the treatment of six critically ill or mechanically-ventilated patients, resulting in reduced endothelial damage and inflammation. Recipients showed an increased survival rate and improved inflammatory markers, including circulating endothelial cells, IL-6, IL-8, CRP, and LDH[107]. Common side effects include headache, upper respiratory infection, fatigue, nausea, vomiting, diarrhea, hypokalemia, neutropenia and fever[108,109]. A recently identified variant in the MBL gene 2 (rs1800450) has been associated with the need for hospitalization, severe disease, ICU admission, and development of pneumonia potentially suggesting a new therapeutic target[41,42].

Other potential targets include antibodies against SARS-CoV-2, such as nCoV396, a monoclonal antibody against the SARS-CoV-2 nucleocapsid (N) protein that has been shown to prevent the MASP-2-dependent complement activation. Binding of nCoV396 to the SARS-CoV 2 N protein leads to conformational changes that may lead to allosteric modulation of its protein function[110]. The precise interaction between the SARS-CoV-2 N protein and MASP2 remains under investigation. Overall, targeting N protein may be a feasible therapeutic strategy.

Given the fact that only a small proportion of patients will develop aggressive disease, reliable clinical indicators to identify these patients in the early phase of disease progression are of utmost importance. The time window for optimal intervention and the patient populations that could benefit from therapeutic complement inhibition have yet to be determined. Currently available biomarkers of complement activity are too unstable and short-lived to be used predictively. Nevertheless, clinical

predictors of ARDS progression combined with inflammatory biomarkers (CRP, IL-6, ferritin, and D-dimer) could potentially allow the identification of patients that could benefit from early intervention[2, 111].

Theoretically, upstream targets in the complement pathway would provide the most potent anti-inflammatory results[96]. Despite the fact that the use of anti-C5a antibodies has been associated with prominent clinical improvement and decreased systemic inflammation, C5 inhibition can be partial, allowing residual terminal pathway activity in cases of excessive complement activation, as seen in severe COVID-19. In these advanced stages of COVID-19, C3 inhibition has the ability to control both ARDS and the systemic inflammation that damages the microcirculation of vital organs. Proximal complement inhibitors which target C3 or its upstream activators are appealing targets, but their benefit in mortality was not confirmed in a randomized, double-blinded, multicenter study that compared APL-9 (C3 inhibitor) to standard of care in mild to moderate COVID-19[112]. Further randomized studies comparing different complement inhibitors are necessary to identify the most appropriate therapeutic agents, as well as the benefits of upstream inhibition or pathway specific targeting.

The available data should be interpreted with caution. Concurrent use of antiviral drugs, corticosteroids, heparin, and antibiotics in these studies significantly limits their generalizability. The increased risk of opportunistic infections, most notoriously with encapsulated organisms (*Neisseria*, *Haemophilus,* or *Streptococcus* species) in unvaccinated individuals and those with asplenia or functional asplenia, through the inhibition of terminal complement proteins has historically limited complement inhibitor use. However, growing clinical experience with C5-inhibitors and C3-inhibitors such as APL-2 and AMY-101/Cp40 along with prophylactic antibiotics or planned vaccination schedules has assuaged these concerns. Additionally, individualized treatment strategies based on specific immunologic profiles and complement-driven disease should be further investigated[113].

The complement system can also be theoretically exploited alongside the use of COVID-19 vaccines and antibody-based therapies. Complement activation is known to enhance the efficacy of pathogen-neutralizing antibodies through formation of larger antibody-C1q complexes, and thus may require fewer IgG molecules bound to virus surfaces to facilitate their neutralization[114,115]. Monoclonal antibodies or vaccines can potentially be engineered to promote enhanced C1q binding and complement activation leading to a more robust immunologic response, confronting the problem of waning antibody concentrations with traditional immunization approaches[115,116]. The risk of vaccine-induced thrombotic thrombocytopenia seen with the use of COVID-19 adenovirus-vector vaccines that is thought to be mediated by anti-platelet factor 4 antibodies and subsequent complement activation remains a concern[117]. Thus, a careful weighing of risks is essential.

CONCLUSION

Current evidence suggests excessive complement activation and subsequent complement-dependent cytotoxic tissue damage drives COVID-19 progression and thromboembolic complications. In the context of thromboinflammation, the three complement pathways can activate the coagulation cascade causing TMA and end-organ damage, mostly manifesting as lung, kidney, and cutaneous disease. Considering its role in cytokine storm and thrombogenesis, the complement system is an appealing treatment target. Preliminary reports have produced promising results. Whether inhibition of upstream (C3, C1) or terminal (C5, C5a, or C5aR) components is of greater importance remains to be elucidated. Current data indicate the need for evaluation of complement inhibitors as COVID-19 therapeutics, and many are under investigation in prospective randomized trials. Limitations such as the cost of inhibitors or their association with opportunistic infections may preclude their generalized use in the treatment of COVID-19.

ACKNOWLEDGEMENTS

FOOTNOTES

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