Review

# Complement activation in COVID-19 and targeted therapeutic options: A scoping review

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## ABSTRACT

Increasing evidence suggests that activation of the complement system plays a key role in the pathogenesis and disease severity of Coronavirus disease 2019 (COVID-19). We used a systematic approach to create an overview of complement activation in COVID-19 based on histopathological, preclinical, multiomics, observational and clinical interventional studies. A total of 1801 articles from PubMed, EMBASE and Cochrane was screened of which 157 articles were included in this scoping review. Histopathological, preclinical, multiomics and observational studies showed apparent complement activation through all three complement pathways and a correlation with disease severity and mortality. The complement system was targeted at different levels in COVID-19, of which C5 and C5a inhibition seem most promising. Adequately powered, double blind RCTs are necessary in order to further investigate the effect of targeting the complement system in COVID-19.

## 1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to another epidemic caused by a coronavirus. Coronavirus disease 2019 (COVID-19) has resulted in over six million deaths globally and has led to substantial morbidity [1,2]. Clinical manifestation ranges from asymptomatic to severe, with development of acute respiratory distress syndrome (ARDS) and multiorgan failure [1,3,4]. Mortality rates in severe COVID-19 remain high due to multiorgan failure inflicted by uncontrolled inflammation [5,6], even with SARS-CoV-2 vaccinations [7,8]. The complement system plays an important role in the innate immune response by marking pathogens, mediating lysis and attracting inflammatory cells to the infection site [9]. Increasing evidence suggests a key role for activation of the complement system in the pathogenesis and disease severity of COVID-19 [10–16].

### 1.1. Complement system

The complement system can be activated through three different pathways; the classical pathway, the lectin pathway, also known as the mannose-binding lectin (MBL) pathway, and the alternative pathway (Fig. 1) [17]. Via the classical pathway, foreign microorganisms are marked by binding of antibodies to antigens, which leads to further complement activation, production of potent anaphylatoxins and formation of the membrane attack complex (MAC) [18]. The MAC plays a crucial role in host defense through cell lysis [19,20]. The lectin pathway employs receptors which have the ability to recognize pathogens and distinguish them from host cells, whereas the alternative pathway is able to recognize and eliminate pathogens without requiring antibodies or prior contact with a pathogen. Furthermore, inhibitory proteins of the alternative pathway prevent aberrant complement activation [18,21].

Activation of the classical pathway begins when C1q, a

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subcomponent of C1, binds to immune complexes [17,19]. Complement proteins from the alternative pathway are called factors and are followed by a capital letter, such as factor B and factor D. The MBL pathway consists of MBL itself, two MBL-associated serum proteases 1 and 2 (MASP-1 and -2) and recognition molecules ficolin-1, −2 and − 3, which are involved in the activation of the MBL pathway [22]. The MBL and alternative pathway can be activated by the binding of C3b, MBL, ficolins or properdin with carbohydrate structures on injured cells or microorganism-associated molecular patterns [19]. The terminal part of the complement pathway is the MAC, consisting of activated protein C5 and complement components C6-C9 (C5b-9) [19]. Activation of one or more pathways by pathogens eventually leads to the activation of C3 and C5 convertases that cleaves key components C3 and C5 into bioactive components C3a, C3b, C5a and C5b. These components modulate the formation of the MAC [23]. Furthermore, C3b is able to bind to the pathogen and continues, or even amplifies, the complement cascade. The formation of the anaphylatoxins C3a and C5a leads to the release of cytokines and causes inflammation at the infection site resulting in tissue injury. In addition, C3a and C5a cause activation of the coagulation system through expression of tissue factor by neutrophils and endothelial cells [24–29]. Not only can C5a be generated through the conventional complement pathways, but also through direct cleavage of C5 by various enzymes such as thrombin, trypsin, and plasmin [30,31]. On the one hand, the role of the complement system in the host defense against invading microorganisms is vital, but on the other hand the harmful effects that come with its hyperactivation stresses the importance of its tight regulation [17].
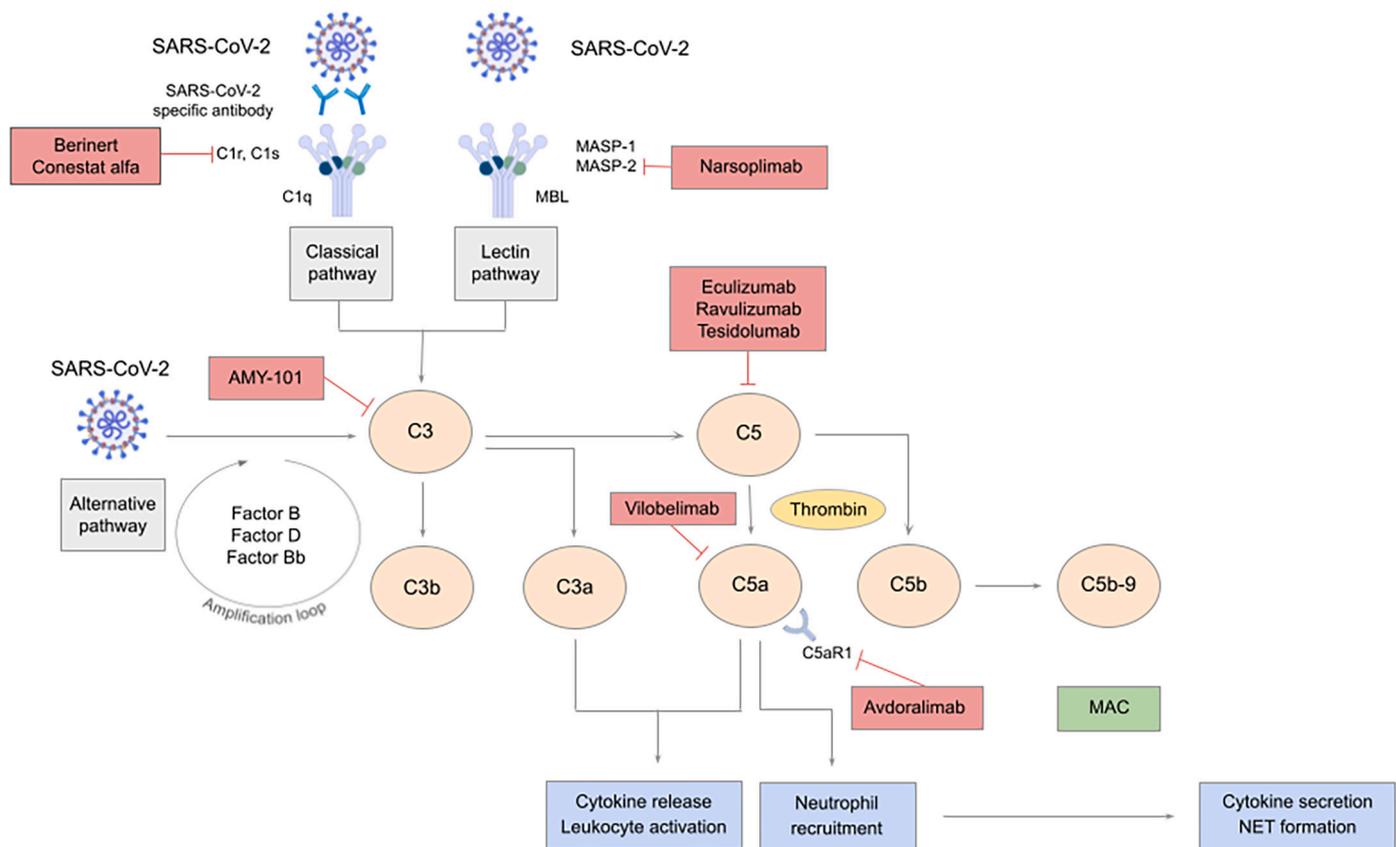
### 1.2. Complement in COVID-19

In COVID-19, the complement system can be activated through all three complement pathways [13,32,33]. However, given the ongoing thrombotic events in COVID-19, complement activation can also occur by enzymes such as thrombin, trypsin and plasmin [30,31]. It has been shown that complement is directly activated by SARS-CoV-2 through the MBL pathway via MASP-1 and MASP-2 and that the SARS-CoV-2 spike protein directly activates the alternative pathway [34,35]. A multicenter biomarker study found that enhanced activation of the alternative pathway was most prevalent in patients with severe COVID-19 and that this was associated with markers of hypercoagulability such as von Willebrand factor, and markers of endothelial injury such as thrombomodulin and angiopoietin-2, which are characteristic features of severe COVID-19 [12,13,32]. Furthermore, thromboinflammation by neutrophil extracellular traps (NETs) in COVID-19 has been shown to be C5a/C5aR1 dependent [36].

It has been demonstrated in vitro and in vivo that complement activation is highly present in patients with ARDS and that C5a is associated with the lung injury caused by inflammatory cell influx [13,37–41]. In a small in vivo model, C3 knock-out mice infected with SARS-CoV-1 exhibited significantly less respiratory dysfunction and pathology compared with control mice, thereby identifying complement as an important mediator [42]. High levels of C5a and C5b-9 have been reported in patients with severe COVID-19 and are associated with disease severity and mortality [12,14,29,36,43]. C5b-9 levels were higher in COVID-19 patients with respiratory failure compared with non-COVID-19 respiratory failure [12]. Several autopsy studies with cases of severe COVID-19 infection showed significant deposits of terminal complement components and MASP-2 in the microvasculature of lung and kidney tissue, further strengthening the role of complement activation [23,44–46].

Currently, emerging treatment strategies targeting complement on different targets have been initiated in COVID-19 patients [13]. In this



**Fig. 1.** Simplified figure of the complement system and theurapeutic targets in COVID-19.
MAC = membrane attack complex; MBL = mannose-binding lectin; NET = neutrophil extracellular trap. Adapted from: Afzali et al., Nat Rev. Immunol (2022).

scoping review, we aim to systematically create an overview of complement activation in COVID-19 based on histopathological, preclinical, multiomics, observational and clinical intervention studies.

## 2. Methods

The Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) statement was used as guideline [47]. The review protocol was determined before writing this review, but not published elsewhere.

### 2.1. Information sources, search strategy and selection process

PubMed/Medline, Embase (Ovid) and Cochrane Library were accessed for articles until May 30, 2022. Search terms included COVID-19, SARS-CoV-2 and complement. The complete search strategy can be found in the **Search strategy Box A** in the supplementary materials. Language was restricted to English articles. Three independent reviewers (EL, VdB and RvA) screened the articles by title and abstract using Rayyan [48]. When deemed eligible, the articles were selected for the full text stage by EL and RvA. Reference lists of included studies were manually screened for eligible articles to ensure a comprehensive search. In case of conflict, a fourth reviewer (LvV) was consulted.

### 2.2. Eligibility criteria

The inclusion criteria for human studies were an age of 16 years or older, presumed SARS-CoV-2 infection or diagnosed with SARS-CoV-2 by means of a real time polymerase chain reaction (RT-PCR) or nucleic acid amplification test (NAAT) and available complement measurements or studies targeting the complement system. Case reports or case studies with less than four patients, preprints and abstracts or articles of which the full-text was not available were excluded. Language was restricted to English articles only. Observational and clinical intervention studies in specific subpopulations were limited to disease severity only. Eligible articles were divided into the following categories: 1) histopathological studies 2) preclinical studies 3) omics 4) observational and 5) clinical interventional studies. Articles could be included in multiple categories.

### 2.3. Data collection process

Data was extracted into pre-defined extraction forms by EL and RvA. For each study category a separate table was used describing the characteristics of each study, study design or methods, outcomes and if applicable various clinical outcomes or key findings. Patients were classified as asymptomatic, mild, moderate or severe. Patients on non-mechanical or mechanical ventilation and patient admitted to the intensive care unit (ICU) were considered as severe.

## 3. Results

The search resulted in a total of 2594 eligible articles (**Search strategy Box A** in the supplementary materials). After removal of duplicates, 1801 articles were screened. Full-text screening resulted in 260 articles which were assessed for eligibility (Fig. 2). In total, 16 histological and/or autopsy studies, 23 preclinical studies, 36 omics studies, 81 observational studies and 13 clinical interventional studies were identified. Twelve studies were included in multiple categories. The characteristics and observations of the studies are described in the corresponding (supplementary) tables (Table 1-2, **S1–4**).

### 3.1. Histopathological studies

Several studies involved skin biopsies of COVID-19 patients (**Table S1**) [49–53]. The majority of studies revealed complement
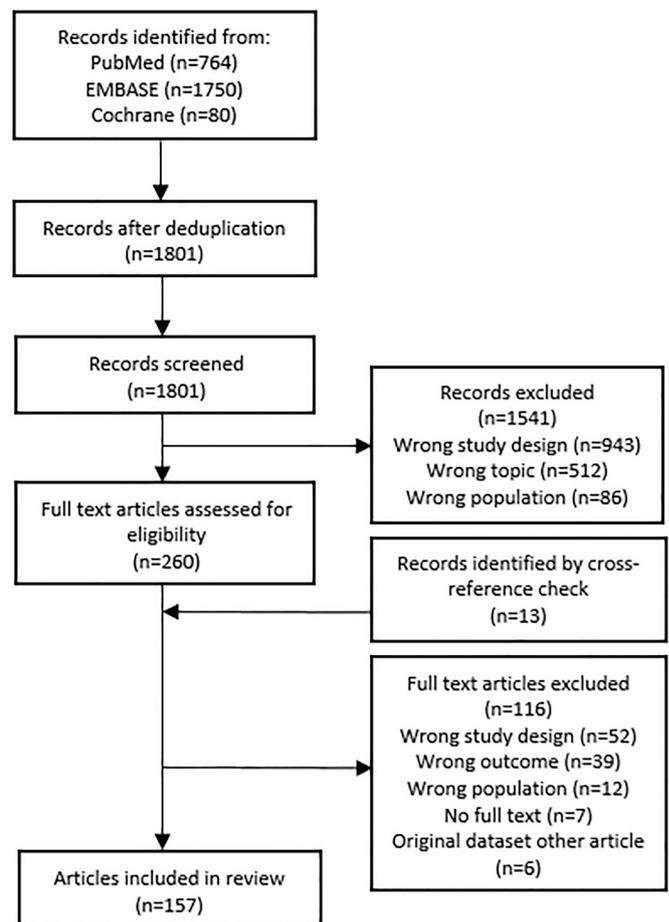


**Fig. 2.** Flow diagram for study selection.

depositions, including C3d, C4d and notably C5b-9, in the vascular system throughout the skin [50,51,53]. Moreover, increased C4d deposition was also seen in and around vessels where fibrin thrombi were located [54]. However, one study reported no complement depositions in the majority of skin lesions [49]. Specific skin lesions, such as chilblain-like skin lesions, also revealed complement activation [52]. Autopsy studies identified complement depositions of all three pathways (C1q, C4, C5b-9, C3, C3d, factor B, Factor D, factor H, MASP-2) in the lungs [45,55,56]. Two studies showed activation of the lectin pathway in the lungs [56,57], however depositions of the lectin pathway were hardly detectable in another study [45]. In parenchyma of lung tissues of critically ill patients, increased T helper (Th)2-biased adaptive immune response was observed with apparent activation of the complement system (C3b and C5b-9) [58]. In the kidneys, cleavage products of C3 were heavily present in renal and glomerular arteries. C5b-9 was observed on the tubules, peritubular capillaries and renal arterioles [44,46]. Components of the lectin and alternative pathway were activated in the kidneys of COVID-19 patients [46]. Deposits of the classical and alternative pathway were found in liver tissue of diseased COVID-19 patients [45,59], albeit C4d deposition was limited and C5d absent in one study measuring only these two components [59]. One study examined heart tissue of deceased COVID-19 patients, in which more C5b-9 was seen compared to non-COVID-19 controls [60].

Overall, histopathological studies showed widespread complement activation in the vascular system throughout the skin, lungs and kidneys.

### 3.2. Preclinical studies

Most preclinical studies concerned cell experiments (Table 1). SARS-

**Table 1**
Summary characteristics of preclinical studies.

| Author & year | Study design | Study population | Model | Methods | Intervention/ complement factors measured | Severity COVID-19 | Key findings |
|---|---|---|---|---|---|---|---|
| Lage [62], 2022 | Experimental monocytes | Monocytes | Isolated monocytes from COVID-19 patients incubated with complement antibodies | Flow cytometry | C1q, C3 | Mild-severe | Increase in C1q and C3 on monocytes from COVID-19 patients compared to healthy controls, which remained elevated even after a short recovery period. No difference between disease severity. |
| Stravalaci [63], 2022 | Experimental | N.A. | SARS-CoV-2 spike protein-coated plates incubated with (complement depleted) serum | Complement deposition assay | C1q-, C4- or C3-depletion | – | SARS-CoV-2 spike protein, by interacting with MBL, activates the complement lectin pathway. |
| Georg [65], 2022 | Experimental cell | T cells | Mechanistic studies on T cells exposed to COVID-19 serum | Flow cytometry, specific T cell cultures/assays | Anti-C3a antibody | Severe | SARS-CoV-2 triggers complement activation (C3a) and drives differentiation of T cells with high immunopathogenic potential. Increased generation of C3a in severe COVID-19 induced activated CD16+ cytotoxic T cells. |
| Posch [64], 2021 | Experimental cell | Human airway epithelial cells | HAE or Vero/TMPRSS2 infected with SARS-CoV-2 | – | C3aR and C5aR antagonists | – | Normal human bronchial and small airway epithelial cells respond to infection by local C3 mobilization, intracellular complement activation, destruction of the epithelial integrity and secrete high levels of C3a. Targeting C3aR and C5aR can prevent intrinsic lung inflammation and tissue damage from SARS-CoV-2. |
| Kovacs-Kasa [69], 2022 | Experimental cell | HLMVEC | Endothelial permeability measurement of HLMVEC exposed to plasma from SARS-CoV-2 patients | ECIS | C3aR and C5aR antagonists | Non-severe, severe | SARS-CoV-2 induced permeability is not affected by C3a or C5a inhibitors. |
| Perico [68], 2022 | Experimental | HMEC-1 | Endothelial cells exposed to SARS-CoV-2 derived spike protein 1 | Immunofluorescence | C3aR and C5aR antagonists | Severe | Endothelial dysfunction induced by SARS-CoV-2-derived S1 protein triggers exuberant complement deposition on activated microvascular endothelial cells C3a and, to a lesser extent, C5a, further amplify complement activation that fuels inflammation in response to S1. |
| Zhang [66], 2021 | Experimental cell | Neutrophils and HUVECs | Neutrophils exposed to plasma from HCs or COVID-19 patients and HUVECs exposed to supernatant of neutrophils cultured with COVID-19 plasma | MPO-DNA, cell viability assay | Anti-C3a and anti-C5a antibodies | Mild or severe | Anaphylatoxins C3a and C5a in the plasma of COVID-19 patients strongly induced NET formation, which could be relieved by CPB. Cell viability of HUVEC is reduced after exposure to plasma from mild and severe COVID-19 patients compared to HDs, which could be reduced by CPB or anti-C3a antibody plus anti-C5a antibody. |
| Apostolidis [67], 2022 | Experimental platelets | Platelets | Platelets from COVID-19 patients, activation, neutralization and inhibition assays | Flow cytometry | Anti-C3a and anti-C5a antibodies | Hospitalized | C5a-C5aR pathway, and weakly C3a-C3aR pathway, mediates hyperactivity in platelets driven by COVID-19 plasma. |
|  | Experimental cell | Neutrophils and monocytes | C5aR measured in neutrophils and | – | – | – | C5aR1 is highly expressed on myeloid cells and |

*(continued on next page)*

**Table 1** (*continued*)

| Author & year | Study design | Study population | Model | Methods | Intervention/ complement factors measured | Severity COVID-19 | Key findings |
|---|---|---|---|---|---|---|---|
| Carvelli [14], 2020 | | | monocytes in peripheral blood from COVID-19 patients and HCs | | | | promotes inflammation in COVID-19 patients. |
| Skendros [36], 2020 | Experimental cell | Neutrophils and HAEC | Neutrophils stimulated with PRP from patients with COVID-19 and HAECs exposed to either COVID-19 derived PRP or NETS (generated in vitro by exposure to PRP from patients with COVID-19) | MPO-DNA, TAT complex, immunofluorescent staining | C5aR1-antagonist | Moderate-severe | C5aR1 blockade attenuated platelet-mediated NET-driven thrombogenicity. COVID-19 serum induced complement activation in vitro. C3 inhibition disrupted tissue factor expression in neutrophils. |
| Aiello [70], 2022 | Experimental cell | HMEC-1 | HMEC-1 exposed to COVID-19 serum | Immunofluorescence | C5aR1 antagonist | Severe | HMEC-1 exposed to COVID-19 serum exhibited significantly higher C5b-9 formation on the cell surface than control serum. Perfusion with whole blood on HMEC-1 pre–exposed to COVID-19 serum resulted in platelet adhesion and aggregation, addition of C5a receptor antagonist fully prevented it. |
| Yu [16], 2021 | Experimental cell | TF1PIGAnull cells | TF1PIGAnull cells exposed to serum of COVID-19 patients | Modified Ham test, flow cytometry | Anti-C5 antibody or factor D inhibitor | Moderate-severe | Serum from COVID-19 patients can induce complement-mediated cell death and increase C5b-9 deposition on the cell surface, which can be mitigated by C5 and factor D inhibition. SARS-CoV-2 spike proteins block complement factor H from binding to heparin. Increased APC activation is associated with COVID-19 disease severity. |
| Yu [35], 2020 | Experimental cell | TF1PIGAnull cells | TF1PIGAnull cells exposed to serum of healthy patients with spike protein S1 and S2 subunits from SARS-CoV-2 | Modified Ham test, flow cytometry | Anti-C5 antibody or factor D inhibitor | – | SARS-CoV-2 spike protein (subunit 1 and 2) directly activates APC. C5 inhibition prevents accumulation of C5b-9 in vitro in response to SARS-CoV-2 spike proteins. |
| Lam [72], 2021 | Experimental red blood cell isolation | Red blood cells | Isolated red blood cells from patients incubated with complement antibodies | Flow cytometry, ELISA | (Anti-) C3b/ iC3b/C3dg and C4d antibodies | Severe | SARS-CoV-2 infection leads to complement activation in vivo. Enhanced C3b and C4d depositions on erythrocytes in COVID-19 sepsis patients compared with healthy controls increased further on day 7, supporting the role of complement in sepsis-associated organ injury. Erythrocytes could help in identifying patients who may benefit from complement targeted therapies. |
| Ali [34], 2021 | Experimental cell | Transfected HEK-293 cells expressing SARS-CoV-2 S protein | HEK-293 cells transfected with SARS-CoV-2 proteins and SARS-CoV-2 proteins incubated with complement antibodies | FACS, ELISA | Anti-C3c, anti-C3b, anti-C4b, anti-C4c and MASP-2 inhibitor | – | SARS-CoV-2 proteins bind to recognition molecules of the LP with subsequent activation of C3b and C4b. MASP-2 inhibitor blocks LP-mediated complement activation. |
| Savitt [61], 2021 | Experimental SARS-CoV-2 proteins | – | SARS-CoV-2 proteins incubated with complement or healthy serum | ELISA | Anti-C1q, anti-C4d antibodies | – | SARS-CoV-2 proteins bind C1q and activates the classical pathway of complement, bind to gC1qR which in turn could serve as |

**Table 1** (*continued*)

| Author & year | Study design | Study population | Model | Methods | Intervention/ complement factors measured | Severity COVID-19 | Key findings |
|---|---|---|---|---|---|---|---|
| Freda [158], 2021 | Experimental cell | Human aortic adventitial fibroblasts | Incubation of AFs with SARS-CoV-2 proteins | ELISA | Anti-gC1qR antibody | – | a platform for the activation of the complement system. After incubation, the expression of gC1qR, ICAM-1, tissue factor, RAGE and GLUT-4 was significantly upregulated. In general, the extent of expression was different for each SARS-CoV-2 protein, suggesting that SARS-CoV-2 proteins interact with cells through different mechanisms. |
| Kisserli [73], 2021 | Experimental red blood cell | Red blood cells | Assessing CR1 density and levels of C3b/C3bi and C4d deposits on erythrocytes | Flow cytometry and PCR amplification | CR1, C3b/C3bi and C4d antibodies | Severe | Decrease in CR1/E and presence of C4d/E deposits confirms the role of complement. Elevated C4d/ E deposition might be an early signal of vascular damage. Complement regulatory molecules could be useful in the treatment of COVID-19. |
| Fernández [71], 2022 | Experimental cell | Human microvascular endothelial cells (HMEC-1) | HMEC-1 incubated with healthy donor, critical COVID-19 or septic shock plasma | Immunofluorescent staining | Anti-C5b-9 antibody | Severe | COVID-19 patient plasma results in similar C5b-9 deposits on endothelial cells as septic shock patient plasma. |
| *Animal studies* | | | | | | | |
| Becker [74], 2021 | Experimental animal | Hamster | Hamsters intranasally infected with 10ˆ5 plaque forming units SARS-CoV-2 | Immunohistochemistry | Anti-C3c antibody | – | Vascular lesions included endothelialitis and vasculitis at 3 and 6 days post infection (dpi), and were almost nearly resolved at 14 dpi. Importantly, virus antigen was present in pulmonary lesions, but lacking in vascular alterations. In good correlation to these data, NETs were detected in the lungs of infected animals at 3 and 6 days post infection. Strong C3c signals are present in inflamed lung tissue 3 and 6 days after infection. |
| Nuovo [75], 2020 | Experimental animal | Mice | Mice intravenous injected with spike peptides of SARS-CoV-2 (without infectious virus) | Immunohistochemistry | C5b-9 antibody | – | Endothelial cell damage with increased C5b-9 (caspase-3, ACE2, IL6, TNFa) expression was seen in the microvessels of the skins and brain in the group with co-localization with the S1 spike protein. |
| Aid [76], 2022 | Experimental animal | Hamster | Hamsters receiving vaccination or sham and challenged intranasally with SARS-CoV-2 | Immunohistochemistry, transcriptomic profiling (RNA-seq) | SARS-CoV-2 vaccination (Ad26.COV2·S) | – | Unvaccinated hamsters showed significant upregulation of complement activation, mainly in C3, C7 and C2. Vaccinated hamsters showed significant downregulation of complement activation. |
| Aid [76], 2022 | Experimental animal | Macaque | Macaques receiving vaccination or sham and challenged intranasally with SARS-CoV-2 | Immunohistochemistry, proteomic profiling | SARS-CoV-2 vaccination (Ad26.COV2·S | – | Markers of the complement cascade (C6, C2, C3, CFB) were increased in sham unvaccinated compared to vaccinated macaques. |

**Abbreviations:** ACE = angiotensin-converting enzyme; AF = human aortic adventitial fibroblast; APC = alternative pathway of complement; CPB = carboxypeptidase B; ECIS = electric cell-substrate impedance sensing; ELISA = enzyme-linked immunosorbent assay; FACS = fluorescence-activated single cell sorting; HAE = Human airway epithelia; HAEC = human aortic endothelial cell; HC = healthy control; HD = healthy donor; HFNO = high flow nasal oxygen; HLMVEC = human lung microvascular endothelial cells; HMEC-1 = human microvascular endothelial cells-1; HUVEC = human umbilical vein endothelial cells; ICU = intensive care unit; IL = interleukin; LP = lectin pathway; MASP = mannose-binding protein-associated serine protease; MPO = myeloperoxidase; NETs = Neutrophil extracellular traps; PCR = polymerase chain reaction; PRP = platelet-rich plasma; seq = sequencing; TAT = thrombin-antithrombin; TMPRSS2 = transmembrane serine protease 2; TNFa = tumor necrosis factor alpha.

CoV-2 proteins were shown to bind C1q in an experimental study with SARS-CoV-2 proteins, as well as recognize molecules of the lectin pathway with activation of C3b and C4b in cell studies [34,61]. In monocytes of COVID-19 patients, an increase was seen in C1q and C3 compared to healthy controls [62]. Other cell studies showed that the alternative pathway was directly activated by SARS-CoV-2 proteins and was associated with disease severity [16,35,63]. Inhibition of MASP-2 by using an inhibitory monoclonal antibody was shown to block complement activation mediated by the lectin pathway in experimental cells [34]. Normal human bronchial and small airway epithelial cells reacted to SARS-CoV-2 infection with local mobilization of C3, intracellular complement activation, destruction of epithelial structure and secretion of high levels of C3a [64]. Inhibition of C3 with compstatin Cp40 hindered expression of tissue factor in neutrophils [36]. An experimental study in T cells showed that C3a activation was triggered by SARS-CoV-2 and led to differentiation of T cells with high immunopathogenic characteristics [65]. C3a and C5a in plasma of COVID-19 patients led to the induction of NET formation and recombinant carboxypeptidase B was shown to alleviate damage of vascular cells by decreasing C3a- and C5a-induced NET production [66]. One experimental cell study in neutrophils and monocytes showed that C5aR1 is abundantly expressed on myeloid cells leading to inflammation in COVID-19 patients [14], and another experimental cell study in neutrophils and human aortic endothelial cells showed that blockade of C5aR1 with C5aR1 antagonist C5aRa/PMX-53 impairs platelet-mediated NET-driven thrombogenicity [36]. In platelets from COVID-19 patients, the C5a-C5aR pathway mediates hyperactivity driven by COVID-19 plasma [67]. Inhibition of C3aR (SB 290157) and C5aR (mix of W-54011 and DF2593) in human airway epithelial cells led to mitigation of viral infection and a decrease of the inflammatory response in airways, whereas inhibition of C5aR also maintained the epithelial integrity of human airway epithelia infected with SARS-CoV-2 [64]. One experimental study in human microvascular endothelial cells (HMEC-1) showed that endothelial dysfunction induced by SARS-CoV-2 derived S1 protein triggered complement deposition on activated microvascular endothelial cells [68]. In particular C5a further amplified activation of the complement system, which contributed to inflammation in response to S1 [68]. However, in one experimental cell study, pulmonary microvascular endothelial cell permeability induced by SARS-CoV-2 was not affected by C3aR (SB290157) and C5aR (W54011) antagonists [69]. HMEC-1 exposed to COVID-19 serum showed significantly higher C5b-9 formation of the cell surface compared with control serum [70]. C5aR1 antagonist (CCX168) completely prevented platelet adhesion and aggregation, which arose when perfusion with whole blood on HMEC-1 pre-exposed to COVID-19 serum was performed [70]. An increase in C5b-9 deposits on human microvascular endothelial cells was associated with COVID-19 severity, but did not differ from septic shock patients [71]. C5 and factor D inhibition with anti-C5 monoclonal antibody (anti-C5Ab) and factor D inhibitor (ACH145951) respectively, was shown to hamper accumulation of C5b-9 induced by SARS-CoV-2 proteins in two experimental cell studies [16,35]. Two experimental red blood cell studies showed complement depositions of C3d, C4d and a decrease of complement receptor 1 (CR1) on erythrocytes, indicating a role of complement activation in COVID-19 [72,73].

We identified a few animal studies on the complement system and COVID-19. Hamsters infected with SARS-CoV-2 showed vascular lesions including endothelialitis and vasculitis, NETs and immunohistochemistry showed strong C3c staining in inflamed lung tissue compared with controls [74]. Mice injected with the SARS-CoV-2 S1 spike protein showed endothelial cell damage with increase of immunohistochemical C5b-9 expression in the microvessels of the skin and brain compared with mice injected with the S2 subunit [75]. Complement activation was significantly decreased in hamsters and macaques vaccinated with Ad26.COV2·S compared with unvaccinated hamsters and macaques [76].

Taken together, preclinical studies showed evidence of extensive complement activation of SARS-CoV-2 through all three pathways. These studies investigated potential targets of the complement system in COVID-19 and showed the implications of targeting these targets.

### 3.3. Omics

Transcriptomic studies identified that genes functioning in the complement pathways are significantly upregulated in COVID-19, notably in severe disease and endures over time (**Table S2**) [77–81]. A transcriptomic study determined RNA levels of 28 complement genes of previously analyzed whole-blood transcriptomic data from 32 patients with different severity of COVID-19 [78]. A variety of genes involved in the complement pathways were expressed, where expression of classical pathway genes was increased in patients with moderate disease, while expression of increased lectin and alternative pathway genes was increased in patients with severe disease and correlated with biomarkers of inflammation and coagulopathy. In particular, C3 was upregulated in several studies and was associated with ICU hospitalization or severity [78,79,82–85]. Most proteomic studies identified complement proteins and regulatory factors as a key component in the immunologic reaction in response to COVID-19 [86–91]. A proteomic study identified the interaction of complement system proteins with the SARS-CoV-2 spike glycoprotein in plasma from 123 COVID-19 patients with different severities [88]. The classical and alternative pathway were an essential component in the overreaction of the immune system in response to COVID-19. MBL and pentraxin-3, which are activators of the complement pathway, were associated with mortality. Multiomics confirmed the upregulation of complement activation on both proteomic and transcriptomic level and the association with disease severity [92–96]. Bioinformatic studies showed upregulation of complement activation [97–99].

In conclusion, multiomics showed upregulation of the complement pathways and associations with disease severity and mortality.

### 3.4. Observational studies

Most observational studies were cohort studies (**Table S3**). 67 of 81 (83%) studies showed increased complement activation in COVID-19 patients. C3 and C4 were most often measured and were generally either decreased in severe patients or non-survivors [100–105], or did not differ compared with non-severe patients or survivors [106–118]. Anaphylatoxins C3a and notably C5a were elevated and correlated with disease severity, ICU admission and mortality [12,14,65,68,85,119–128]. Soluble C5b-9 levels were also increased and correlated with C5a, markers of inflammation and coagulation [12,33,36,68,70,71,78,120,125,129–134]. C5 was only elevated in three studies measuring C5 levels [66,135,136]. MBL levels in plasma were in general higher in COVID-19 patients compared with healthy controls [134,137,138]. Overactivation of the alternative pathway was observed as well, especially in critically ill COVID-19 patients [12,16,43,122,139]. In a prospective cohort study of 219 hospitalized COVID-19 patients, including those admitted to the ICU, increase in concentrations over time of C3a, C5a and factor Bb were associated with death [43].

Summarized, the majority of observational studies showed complement activation in COVID-19 patients. C3a and notably C5a and C5b-9 were increased and correlated with disease severity.

### 3.5. Clinical interventional studies

We identified 13 studies on clinical interventions in the complement system (Table 2, **Table S4a** and **S4b**). Six (46%) were case series, three (23%) were cohort studies and four (30%) were clinical trials. Only two (15%) of these trials were randomized clinical trials (RCTs) and both were phase 2, open-label RCTs. The majority of clinical studies targeted C5, of which four were small case series. Other targets of the

**Table 2**
Summary characteristics of clinical interventional studies.

| Author & year | Study design | Setting | Follow-up duration | Study population & sample size | Control group & sample size | Severity COVID-19 | Therapy, dosage, interval | Concomitant medication | Targeting at what complement level | Main outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| Rambaldi [140], 2020 | Case series | ICU | Until discharge | COVID-19 patients with ARDS ($n = 6$) | Healthy controls ($n = 5$), COVID-19 controls ($n = 33$) | Severe | Narsoplimab 4 mg/kg intravenously twice weekly for 2–4 weeks | Azithromycin prophylaxis (100%), heparin, hydroxychloroquine, darunavir/cobicistat, methylprednisolone | MASP-2 | Narsoplimab treatment was associated with rapid and sustained reduction of circulating endothelial cell count and concurrent reduction of serum IL-6, CRP and LDH. |
| Urwyler [141], 2020 | Case series | Ward | Until discharge | COVID-19 patients with progressive disease after 24 h, CRP >30 mg/L, saturation < 93% (n = 5) | COVID-19 controls during the same period ($n = 15$) | Moderate-severe | Conestat alfa 8400 IU followed by 3 additional doses of 4200 IU in 12 h intervals over 48 h | Hydroxychloroquine (100%), lopinavir/ritonavir (100%), tocilizumab, amoxicillin/clavulanic acid | C1-esterase | Conestat alfa over 48 h was well tolerated and associated with improvement in the clinical condition of 4 patients. No significant difference in length of stay in days, intubation, death, both intubation or death. C4d and C5a decreased within 5 days in most patients. |
| Mansour [142], 2021 | Open-label, phase 2 RCT | Ward, ICU | 28-days | COVID-19 pneumonia, SpO2 ≤ 94% or P/F ratio ≤ 300 mmHg (iC1e/K, $n = 10$; icatibant, $n = 10$) | Randomized controls (n = 10) | Severe | iC1e/k group: Berinert dosage of 20 IU/kg body weight on days 1 and 4. Icatibant group: Icatibant dosage 30 mg 8 h intervals for 4 days | Antibiotics, antithrombotic therapy, corticosteroids | C1-esterase | Neither icatibant nor inhibitor of C1 esterase/kallikrein resulted in changes in time to clinical improvement. However, both compounds were safe and promoted the significant improvement of lung computed tomography scores and increased blood eosinophils. |
| Mastellos [143], 2020 | Cohort study | Ward, ICU | After discharge | Severe COVID-19 patients (AMY-101, n = 3; eculizumab, n = 10) | – | Severe | AMY-101 group: AMY-101 5 mg/kg/daily IV for 9, 12 or 14 days. Eculizumab group: eculizumab 900 mg IV once a week (1–3 doses in total) | Antibiotics (100%), penicillin & corticosteroids (all eculizumab patients), anticoagulants | C3 & C5 | C3 and C5 inhibition elicit an anti-inflammatory response. Mortality in the AMY-101 group was 0/3 (0%) and in the eculizumab group 2/10 (20%). C3a and C5b-9 decreased at day 7 in the AMY-101 group. In the eculizumab group, C5b-9 was increased on day 7. Factor B was decreased at day 7 in the eculizumab group. |
| Diurno [159], 2020 | Case series | Sub-ICU | Until discharge | COVID-19 patients with severe pneumonia or ARDS ($n = 4$) | – | Severe | Up to 4 weekly infusions of eculizumab 900 mg | Heparin, lopinavir/ritonavir, hydroxychloroquine, ceftriaxone | C5 | All four patients successfully recovered after treatment with eculizumab. Mean CRP levels dropped from 14.6 to 3.5 mg/dl and the mean duration of the disease was 12.8 days. |
| Pitts [160], 2021 | Case series | ICU | Until discharge | COVID-19 patients requiring mechanical ventilation due to ARDS (n = 5) | – | Severe | Eculizumab IV 900 mg | Prophylactic antibiotics (100%), hydroxychloroquine, steroids | C5 | 60% mortality rate in patients receiving eculizumab therapy. No deaths were deemed likely study related. |
| Zelek [132], 2020 | Case series | ICU | Until discharge | COVID-19 patients requiring intensive care and ventilation support (n = 5) | – | Severe | Single 1500 mg IV dose of LFG316 (tesidolumab) | Hydrocortisone, antibiotic prophylaxis (phenoxymethylpenicillin or clarithromycin) | C5 | Mortality of 20% (n = 1). In four of five patients, there was sustained improvement in clinical state. In all patients, CP hemolytic activity was completely suppressed up to day 4 after treatment with |

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**Table 2** (*continued*)

| Author & year | Study design | Setting | Follow-up duration | Study population & sample size | Control group & sample size | Severity COVID-19 | Therapy, dosage, interval | Concomitant medication | Targeting at what complement level | Main outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | partial recovery at day 7; C5b-9 and C5a levels fell within the normal range and remained low through day 7. C5 levels did not decrease. |
| De Latour [131], 2020 | Case series | Ward, ICU | Until discharge | Patients with severe pneumonia requiring oxygen ($\geq$ 5 L/min) or mechanical ventilation ($n$ = 8) | – | Severe | Eculizumab injection dosage varied from 900 to 1200 mg every 4 or 7 days (1–5 doses) | Heparin, dexamethasone, prophylactic antibiotics (100%) | C5 | All eight patients were particularly severe at the time of eculizumab initiation and six improved significantly. C5b-9 decreased significantly in the patients treated with eculizumab. |
| Ruggenenti [144], 2021 | Retrospective cohort | Not specified | Until discharge | COVID-19 patients CPAP ventilator support from $\leq$24 h (n = 10) | Contemporary controls ($n$ = 65) | Severe | 900 mg eculizumab IV <24 h of CPAP ventilator support and 7–10 days after the first dose | Hydroxychloroquine, darunavir/ cobicistat, low dose steroids, heparin, ceftriaxone and azithromycin | C5 | Eculizumab was associated with a significant reduction in respiratory rate at one (and two) weeks. Four of the ten eculizumab-treated patients (40%) died or were discharged with chronic complications as compared to 52 of the 65 controls (80%). Event rate was significantly lower in eculizumab-treated patients than in controls. C5b-9 levels significantly decreased after the first dose versus baseline, but not compared with the control group. |
| McEneny-King [145], 2021 | Cohort study | Not specified | 29-days | COVID-19 patients requiring ventilation (invasive or non-invasive) ($n$ = 22) | – | Severe | Ravulizumab dosage weight based (900–3900 mg) on days 1, 5, 10 and 15 | Not reported | C5 | In all patients and at all individual time points after the first dose was administered, ravulizumab concentrations remained >175 µg/mL and free C5 concentrations remained <0.5 µg/mL. Complement plasma level of C5 decreased in all patients treated with ravulizumab |
| Giudice [146], 2020 | Non-randomizedcontrolled trial | ICU | Until discharge | COVID-19 pneumonia or ARDS ($n$ = 7) | Non-randomized controls (n = 10) | Severe | Ruxolitinib 10 mg/BID for 14 days, eculizumab 900 mg IV at day 0, day 7 and when needed day 14 | Azithromycin (100%), heparin, hydroxychloroquine, antivirals (darunavir/cobicistat or lopinavir/ ritonavir), low-dose steroids | C5 (and JAK1/ 2) | On day 7, patients on eculizumab and ruxolitinib displayed a significant improvement in PaO$_2$ and P/F ratio compared to the control group, while no differences were observed for FiO$_2$. In addition, subjects on ruxolitinib and eculizumab showed a significant increase in platelet count compared to control group at day 7. |
| | Non-randomized controlled trial | ICU | 28-days | Severe COVID-19 patients with symptomatic bilateral | Non-randomized controls ($n$ = 45) | Severe | Eculizumab 900 mg IV on days 1, 8, 15 and 22. | Heparin, hydroxychloroquine, antivirals (lopinavir-ritonavir, | C5 | At day 15, estimated survival was 83% (95% CI: 70%–95%) |

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**Table 2** (*continued*)

| Author & year | Study design | Setting | Follow-up duration | Study population & sample size | Control group & sample size | Severity COVID-19 | Therapy, dosage, interval | Concomitant medication | Targeting at what complement level | Main outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| Annane [147,148], 2020 | | | | pulmonary infiltrates confirmed by CT or chest X-ray ≤7 days and severe pneumonia, acute lung injury, or ARDS requiring supplemental oxygen at ICU (n = 35) | | | Amendment: 1200 mg on days 1, 4, and 8 and 900 mg on days 15 and 22. Optional doses of 900 or 1200 mg on days 12 and 18 per investigator decision. | remdesivir), corticosteroids, vaccination and prophylactic cefotaxime against meningococcal infection | | with eculizumab and 62% (95% CI: 48%–76%) without eculizumab, which differed significantly. TESAE of an infectious complication at day 28 was significantly greater with versus without eculizumab (57% vs 27%, respectively. Serum C5b-9 levels decreased over time at day 15, whereas C3 and C4 levels remained stable. C5a did not statistically differ between eculizumab treated and eculizumab-free patients at day 1 and day 7. |
| Vlaar [31,149], 2020 | Open-label, phase 2 RCT | ICU, intermediate care unit, COVID-19 ward | 28-days | Severe COVID-19 pneumonia (pulmonary infiltrates consistent with pneumonia, a clinical history of severe shortness of breath <14 days, or need for noninvasive or MV; P/F ratio 100–250 mmHg (n = 15) | Randomized controls (n = 15) | Severe | 5–7 doses of vilobelimab 800 mg IV (days 1, 2, 4, 8, 11–13, 15 and 22) | Chloroquine, ganciclovir, azithromycin, heparin | C5a | Vilobelimab appears to be safe in patients with severe COVID-19. At day 5 after randomization, the mean P/F ratio showed no differences between treatment groups. Mortality at day 28 did not differ significantly. The frequency of SAEs were similar between groups and no deaths were considered related to treatment assignment. The secondary outcome results in favor of vilobelimab are preliminary. C5a concentrations were suppressed in the vilobelimab group as compared with the control group, which was maintained on day 8. |

**Abbreviations:** ARDS = acute respiratory distress syndrome; BID = bis in die (twice a day); CI = confidence interval; CP = classical pathway; CPAP = continuous positive airway pressure; CRP = c-reactive protein, CT = computed tomography; FiO$_2$ = fractional inspired oxygen; h = hour; iC1e/K = C1-esterase/kallikrein inhibitor; ICU = intensive care unit; IL-6 = interleukin-6; IL-8 = interleukin-8; IU = international unit; IV = intravenous; LDH = lactate dehydenase; kg = kilogram; L = liter; mg = milligram; min = minute; mL = milliliter; mmHg = millimeter of mercury; P/F = PaO$_2$/FiO$_2$; PaCO$_2$ = arterial partial pressure of carbon dioxide; PaO$_2$ = arterial partial pressure of oxygen; PK/PD = pharmacokinetics/pharmacodynamics; SAE = serious adverse event; TESAE = treatment emergent serious adverse event; µg = microgram.

complement system included MASP-2, C1-esterase, C3 and C5a.

MASP-2 was targeted with narsoplimab in a small case series with six COVID-19 patients with ARDS [140]. Narsoplimab was well tolerated and all patients survived, resulting in a significantly lower mortality compared with two retrospective control groups. C1-esterase was targeted with conestat alfa and Berinert in a small case series and open-label RCT, respectively [141,142]. Both treatments were well tolerated but did not show significant differences in clinical outcomes, compared with the control group. Only one study targeted C3 with AMY-101 in three patients, with no treatment related severe adverse events (SAEs) [143]. One retrospective cohort study of ten patients treated with C5 inhibitor eculizumab showed a significant decrease in the combined endpoint of mortality and discharge with chronic complications compared with the control group [144]. Another cohort study assessing the pharmacokinetics/pharmacodynamics (PK/PD) of C5 inhibitor ravulizumab showed decreased plasma levels of C5 in all treated patients [145]. Two non-randomized controlled trials with eculizumab treatment in severe COVID-19 patients showed a significant improvement in $PaO_2/FiO_2$ (P/F) ratio in the treatment group [146,147], although one trial combined eculizumab with JAK1/2 inhibitor ruxolitinib.

The other trial inhibiting C5 with eculizumab observed a significant improvement in estimated survival at day 15 in patients treated with eculizumab solely. However, the proportion of patients treated with eculizumab experiencing a treatment-emergent serious adverse event (TESAE) of an infectious complication was significantly higher than the control group, despite all eculizumab patients were treated with prophylactic antibiotics against meningococcal infection. Ventilator-associated pneumonia (VAP) was significantly higher as well in the group treated with eculizumab. 50% hemolytic complement (CH50) activity of C5 was decreased at day one after infusion of eculizumab [147], however C5a concentrations at day one and day seven did not differ between the two groups [148]. An open-label, phase RCT targeted C5a with vilobelimab [31]. The primary endpoint of mean P/F ratio five days after randomization showed no significant difference between both groups. Although preliminary and not significant, secondary endpoints of mortality at day 28, estimated glomerular filtration rate, lymphocyte count and lactate dehydrogenase, seemed to be in favor of patients treated with vilobelimab. The incidence of SAEs and infectious complications was similar between the two groups without additional antibiotic prophylaxis. Infections classified as serious were reported in three (20%) of the patients treated with vilobelimab compared with five (33%) patients in the control group. C5a concentrations were suppressed in the vilobelimab group after the first dose compared to the control group, which was maintained on day eight [149].

To summarize, the complement system was targeted at different levels in COVID-19 patients. However, only two RCTs were identified, inhibiting C1-esterase and C5a. C5 inhibition was associated with increased bacterial infections.

## 4. Discussion

To the best of our knowledge, this is the first scoping review with a systematical search of complement activation studies in COVID-19. Histopathological, preclinical studies, multiomics and observational studies have identified complement activation in COVID-19 through all three pathways. Complement activation was associated with disease severity, ICU admission and mortality. Our results stress the important role of the complement system in the pathophysiology leading to organ damage and death in COVID-19.

Histopathological studies showed widespread complement activation in the vascular system throughout the skin, lungs and kidneys. Increased activation product C4d was observed in and around vessels where fibrin thrombi were located [54], in line with complement and NET driven immunothrombosis in COVID-19 [36]. Th2-biased adaptive immune responses, accompanying overt complement activation were observed in lung parenchyma of critically ill patients [58]. Complement deposits in the lungs are evidence for local complement activation and substantiate the role of complement activation the development of SARS-CoV-2 induced ARDS [37,41]. Complement depositions found in kidneys are consistent with kidney injury observed in COVID-19 [10,31,44,150].

Upregulation of complement pathways and their association with disease severity was shown in transcriptomic, proteomic, multiomics and bioinformatic studies. Transcriptomic studies identified upregulation of C3 in particular, which was associated with ICU hospitalization or disease severity [78,79,82–84]. The majority of observational studies measured C3 and C4, and generally, both were either decreased in severe patients or non-survivors [100–105], or did not differ between non-severe patients or survivors [106–118]. This was in line with a meta-analysis that showed lower concentrations of both C3 and C4 in patients with high disease severity or non-survivors, compared with patients with low severity or survivors, indicating complement activation and product consumption [151]. Anaphylatoxins C3a and especially C5a were elevated and correlated with disease severity, ICU admission and mortality [12,14,65,68,85,119–128]. Soluble levels of C5b-9 were also increased and correlated with C5a, markers of inflammation and coagulation [12,33,36,68,70,71,78,120,125,129–132,134]. C5 was only elevated in three observational studies investigating C5 [66,135,136].

Potential targets of the complement system in COVID-19 were investigated in preclinical studies. Inhibition of MASP-2 led to blockade of complement activation mediated by the lectin pathway [34]. C3 inhibition hampered expression of tissue factor in neutrophils [36], whereas recombinant carboxypeptidase B alleviated damage of vascular cells by decreasing NET production induced by C3a and C5a [66]. Targeting C3aR and C5aR, the anaphylatoxin receptors of C3a and C5a, in nonimmune respiratory cells reduced an inflammatory response and subsequent tissue damage [64]. However, in another experimental cell study pulmonary microvascular endothelial cell permeability induced by SARS-CoV-2 was not affected by targeting these receptors [69]. Furthermore, blockade of C5aR1 impaired platelet-mediated (NET-driven) thrombogenicity in experimental cell studies [36,70]. Lastly, inhibition of C5 and factor D hampered accumulation of C5b-9 induced by SARS-CoV-2 proteins [16,35].

Although several clinical studies targeting the complement system in COVID-19 were identified, most had major limitations, including a small sample size, lack of randomization, blinding and a proper control group. Most studies included prophylactic antibiotics in patients treated with complement inhibitors. MASP-2 was only targeted in a small case series with six patients [140]. Targeting C1-esterase in a small case-series and small open-label RCT appeared to be safe, however it did not result in improved clinical outcomes compared with the control group [141,142]. C3 was only inhibited in three patients in a non-controlled study [143]. Two non-randomized controlled trials with eculizumab showed significant improvement in P/F ratio in the treatment group [146,147], although one of these trials combined eculizumab with JAK1/2 inhibitor ruxolitinib thus the effect of eculizumab alone cannot be determined. The other trial observed a significant improvement in estimated survival at day 15 in patients treated with eculizumab solely, though infectious complications and VAPs were seen more often with eculizumab treatment despite additional antibiotic prophylaxis against meningococcal infection [147]. Besides, preliminary serum free eculizumab concentrations, CH50 and serum C5b-9 levels led to a protocol amendment in order to increase dosage and frequency of eculizumab treatment during the study. PK/PD analysis of C5 inhibitor ravulizumab showed decreased plasma levels of C5 [145] and CH50 activity of C5 was decreased in patients treated with eculizumab [147]. Only one study targeted C5a in an open-label, phase 2 RCT [31]. No difference was shown in the primary endpoint of mean P/F ratio five days after randomization was observed, but secondary endpoints including mortality at day 28 seemed to be in favor of vilobelimab treatment, albeit considered preliminary. Baseline C5a concentrations were elevated in

all patients and C5a was suppressed in the vilobelimab group compared with the control group, which was maintained on day eight [149]. The development of avdoralimab, a C5aR1-specific monoclonal antibody, was terminated after disappointing results of a phase 2 RCT performed in patients with different severities of COVID-19 (NCT04371367) [13]. An explanation could be that the proinflammatory effects of C5a are still mediated via the other receptor C5aR2, which binds C5a and its des-arginine form [152].

The evidence of this review points towards a critical role of C5a in severe COVID-19. C5 was elevated in only three observational studies investigating C5 [66,135,136]. An advantage of inhibiting C5a specifically compared with upstream inhibition such as C3 or C5 inhibition, is that the formation of the MAC complex is not affected, which plays an important role in bacterial lysis [20,127]. As was seen in C5 inhibition with eculizumab, the incidence of infectious complications and VAPs were significantly higher with eculizumab treatment despite prophylactic antibiotics against meningococcal disease [147], which was not the case with vilobelimab even without additional antibiotic prophylaxis [31]. A previous study showed that inhibition of C5a requires a specifically targeted inhibition [153], and C5a levels were shown not to be different between eculizumab treated patients and controls at day one and day seven [148]. Additionally, C5a can be generated through direct cleavage of C5 in the absence of C3 by various enzymes such as thrombin, trypsin and plasmin, which could be of substantial importance given the thrombotic complications seen in COVID-19 [30,31].

As mentioned earlier, specifically inhibiting upstream components of the complement pathway may increase the risk of bacterial infections [20,127,147,154]. Recent results of a phase 3 RCT comparing ravulizumab plus best supportive care (BSC) versus BSC only (NCT04369469) show 54 SAEs of infections and infestations in patients treated with ravulizumab compared with only 8 SAEs in the BSC group, while C5a inhibition is not associated with an increased risk of infection [31].

Strengths of this review include the systematic search and comprehensive inclusion of histopathological, preclinical, omics, observational and clinical interventional studies. Since the aim of this review was to create an overview of complement activation in COVID-19 and also taking into account the emerging evidence in COVID-19, a scoping review was deemed most appropriate and thus risk of bias assessment was not performed [47,155]. Most included studies were observational cohort studies and often lacking a control group. Clinical studies were scarce, and the majority were case series of a small group of patients. A proper control group and blinding was often lacking in the clinical studies, which could have led to confounding and bias, and only two randomized trials were performed.

Although in particular downstream inhibition of the complement system seems promising, adequately powered, double blind RCTs are needed to further investigate the effects. However, with the dominance of the Omicron variant and population immunity, less severe COVID-19 disease is seen compared with previous waves, which will make sufficient inclusion of severe COVID-19 patients in large trials more challenging. International collaborations and platforms can be the solution for this. Thereby, conducting clinical trials with the aim of showing superiority of treatment will be difficult due to the use of already proven effective therapies in severely ill COVID-19 patients, such as steroids and anti-interleukin-6 treatment [156,157].

## 5. Conclusions and future directions

In this scoping review, histopathological, preclinical, multiomics and observational studies showed apparent complement activation through all three complement pathways in COVID-19. Complement activation in COVID-19 is correlated with disease severity and mortality. Different drugs targeting the complement system have been studied in COVID-19, of which C5 and C5a inhibition seem most promising. Advantages of inhibiting C5a over C5 are the ability to inhibit C5a specifically as C5a

can be generated indirectly and requires a specifically targeted inhibition, the absence of an association with increased bacterial infections, and lastly, C5a seems to be a key driver in severe COVID-19 disease. However, adequately powered, double blind RCTs are necessary in order to further substantiate these findings.

**Practice points**

- Histopathological, preclinical, multiomics and observational studies showed apparent complement activation through all three complement pathways in COVID-19.
- Complement activation in COVID-19 is correlated with disease severity and mortality.
- Different drugs targeting the complement system have been studied in COVID-19, of which those blocking the final common pathway seem most promising.

**Research agenda**

- Adequately powered, double blind RCTs are necessary in order to further investigate the effect of targeting the complement system in COVID-19.

**Declaration of Competing Interest**

Prof. Vlaar reports personal fees from InflaRx paid to Amsterdam UMC, outside the submitted work. All other authors declare no competing interests.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.blre.2022.100995.

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