Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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The interplay between neutrophils, complement, and microthrombi in COVID-19

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A B S T R A C T

As of the end of 2020, coronavirus disease 2019 (**COVID-19**) remains a global healthcare challenge with alarming death tolls. In the absence of targeted therapies, supportive care continues to be the mainstay of treatment. The hallmark of severe COVID-19 is a thromboinflammatory storm driven by innate immune responses. This manifests clinically as acute respiratory distress syndrome, and in some patients, widespread thrombotic microangiopathy. Neutrophils and complement are key players in the innate immune system, and their role in perpetuating fatal severe COVID-19 continues to receive increasing attention. Here, we review the interplay between neutrophils, neutrophil extracellular traps, and complement in COVID-19 immunopathology, and highlight potential therapeutic strategies to combat these pathways.

Introduction

The coronavirus disease 2019 (**COVID-19**) is caused by the novel severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**) [1]. SARS-CoV-2 can present as asymptomatic infection or it may cause a wide spectrum of disease, ranging from mild upper respiratory tract infection to life-threatening sepsis

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with multiorgan failure [2]. Given its rapid spread, high contagiousness, and lack of specific effective treatment, this pandemic remains a global healthcare challenge [1,3,4].

SARS-CoV-2 displays a complex relationship with the human immune system [5]. It has a unique ability during early infection to inhibit host type I interferon and natural killer cell responses, thereby compromising the body's antiviral defenses and leading to high viral loads in some patients [5,6]. As a result of initial immune evasion, the body then mounts a compensatory hyperinflammatory response to aid in viral clearance. This is characterized by persistent hyperactivation of innate immunity with the overproduction of proinflammatory cytokines; hyperinfiltration of neutrophils, monocytes, and macrophages in lung parenchyma; and extensive epithelial and endothelial damage [5,7,8]. This fulminant inflammatory storm can lead to acute respiratory distress syndrome, and, in some patients, provoke thrombotic widespread microangiopathy, which results in multiorgan failure and death. Neutrophils and complement are key players in our innate immune system, and their roles to potentiate severe COVID-19 continues to receive much attention. Here, we will review potential roles of neutrophils and complement in COVID-19 immunopathology and highlight some potential therapeutic strategies that may target these pathways.

Neutrophil extracellular traps and COVID-19

Neutrophils are the most abundant immune cells in circulation. They act as the first responder against various infections. For decades it was assumed that phagocytosis was the primary mechanism by which neutrophils neutralized pathogens. However, in 2004, Brinkmann and colleagues described another distinct activity of neutrophils, in which neutrophils release sticky extracellular “spider webs” composed of neutrophil-derived chromatin, microbicidal proteins, and mitochondrial remnants [9]. They named this process neutrophil extracellular trap (**NET**) release or NETosis. NETs act to prevent the spread of infections and also use their antimicrobial peptides to kill pathogens [9,10]. By exposing potential autoantigens, such as nucleic acids and modified proteins, NETs may also contribute to autoimmune responses in susceptible individuals [10]. In recent years, it has also been revealed that NETs are prothrombotic [11]. NETs activate platelets and the clotting cascade, and ultimately form an integral part of arterial and venous thrombi [11–15].

Prior knowledge regarding NETs in viral infection

Many viruses can directly trigger NET release [16]. HIV and respiratory syncytial virus (**RSV**) induce NETosis through toll-like receptor-mediated signaling [17,18]. Viruses can also promote NETosis indirectly through triggers such as IL-8, type I interferon, and activated platelets [16]. NETs contribute to antiviral immunity by immobilizing viral particles and inactivating them through the release of antimicrobial molecules such as myeloperoxidase, α-defensin, and cathelicidins [16]. While NETs help contain viral spread, they may also cause damage to the host. For example, NETs promote alveolar-capillary injury in RSV and influenza models [19–21]. High levels of NETs are observed in patients with pneumonia-associated acute respiratory distress syndrome (**ARDS**) [16], where isolated neutrophils have a lower threshold for spontaneous NET release.

NETs in COVID-19

It was recognized early in the pandemic that many patients hospitalized with COVID-19 demonstrate neutrophilia, which predicts critical illness and in-hospital mortality [22–24]. Furthermore, autopsy reports of COVID-19 lung tissues revealed intra-alveolar and capillary neutrophil hyperinfiltration, acute capillaritis with fibrin deposition, and neutrophilic mucositis [25–27]. In April 2020, high levels of NETs in the blood of patients with severe COVID were first reported [28]. This study assessed three markers of NETs [cell-free DNA, myeloperoxidase (**MPO**)-DNA complexes, and citrullinated histone H3 (**Cit-H3**)] in sera of 50 patients hospitalized with COVID-19 as compared to 30 healthy controls [28]. All three markers were significantly elevated in COVID-19 sera, while high levels of NETs were associated with the severest disease [28]. Notably, COVID-19 sera robustly triggers NET release from healthy donor neutrophils [28]. These findings were soon confirmed by independent

groups. In a prospective cohort of 33 patients hospitalized with COVID-19, high levels of MPO-DNA complexes were associated with severe respiratory status and worse clinical outcomes [29]. Upon convalescence, levels of MPO-DNA complexes decreased [29]. Others have also confirmed high circulating NETs among COVID-19 patients and their tendency to tracking with disease severity [30,31].

The first microscopic confirmation of NETs was reported in an autopsy series from New Orleans where degenerative neutrophils with strands of extracellular material that stained weakly positive for DNA were present in COVID-19 lungs [32]. Histological detection of NETs has since been made not only in the pulmonary vasculature, but also in the microvasculature of the kidney and heart [30,31,33]. Notably, the transcriptomic analysis of four COVID-19 patients' lung tissue and bronchial alveolar lavage fluid revealed marked enrichment of NET-associated genes [34]. In summary, several lines of data have supported the presence of NETs in the severest forms of COVID-19 (Table 1).

Table 1
Studies investigating NETs in COVID-19.

Reference	Publication Date	Study Design	Study Population	Main Findings
Zuo [28]	April 24, 2020	Cohort	N = 50	Markedly elevated NETs (cell-free DNA, MPO-DNA, and Cit-H3) were detected in the sera of patients hospitalized with COVID-19. Markers of NETs associated with severe respiratory status. Sera from individuals with COVID-19 triggered NET release from control neutrophils *in vitro*.
Fox [35]	May 27, 2020	Autopsy series	N = 10	First microscopic confirmation of NETs in alveoli.
Middleton [29]	June 29, 2020	Prospective Cohort	N = 33	High level of NETs (MPO-DNA) were detected in COVID-19 blood, where they were associated with worse respiratory status and clinical outcomes. Platelet-derived factors known to trigger NETosis were also elevated. COVID-19 plasma potentiated NET release *in vitro*, which could be attenuated by neonatal NET-inhibitory factor. Autopsies revealed colocalization of PF4 and NETs in pulmonary vessels.
Nicolai [33]	July 22, 2020	Cohort	N = 38	Immunothrombi enriched with neutrophils were seen in COVID-19 lungs. Distinct neutrophil signatures were appreciated in COVID-19, which range from a hypoactive phenotype in patients with intermediate COVID-19 to excessive neutrophil activation in severe COVID-19. Platelets isolated from patients with severe COVID-19 were bound to neutrophils and potentiated NETosis *in vitro*.
Leppkes [30]	July 31, 2020	Cohort	N = 71	High level of NETs (cell-free DNA, MPO-DNA, Cit-H3, and neutrophil elastase-DNA complexes) were appreciated in COVID-19 blood, where they were associated with severe disease. Significantly increased low-density neutrophils that have high propensity for spontaneous NETosis were observed in COVID-19 patients. Autopsy of COVID-19 patients demonstrated the occlusion of pulmonary vessels by aggregated NETs.
Wang [34]	Aug 18, 2020	Cohort	N = 4	Transcriptomic analysis of COVID-19 lung tissue and BAL fluid revealed marked enrichment of NET-associated genes.
Veras [31]	Sep 14, 2020	Cohort	N = 32	High levels of NETs (MPO-DNA) were detected in the blood of COVID-19 patients. SARS-CoV-2 directly induced NETosis through a mechanism that was dependent upon PAD4, effective viral replication, and host cell ACE2.

MPO = myeloperoxidase; Cit-H3 = citrullinated histone H3; PF4 = platelet factor 4; NETs = neutrophil extracellular traps; PAD4 = protein arginine deiminase 4; and BAL = bronchial alveolar lavage.

NET induction in COVID-19

In COVID-19, NETs are likely produced in both direct and indirect fashion. NETs can be induced by incubating SARS-CoV-2 with healthy neutrophils [31]; in this context, the inhibition of either PAD4 or RNA polymerase abrogates NETosis. SARS-CoV-2 utilizes ACE2 receptor and serine protease TMPRSS2 to enter host cells [36], and the blockade of either ACE2 or serine protease activity antagonizes SARS-CoV-2-triggered NETosis [31]. Other studies have suggested that NETs are produced indirectly in COVID-19 through activated platelets. Platelets from patients with severe COVID-19 demonstrate increased adhesion to neutrophils *in vivo*, while triggering NETosis from healthy neutrophils *in vitro* [33]. Furthermore, platelet-derived molecules such as platelet factor 4 (**PF4**) and RANTES, which are known to trigger NETosis, are markedly elevated in COVID-19 patients [37]. Autopsy specimens have revealed the colocalization of PF4 and NETs in the pulmonary vasculature [37]. Considering that severe COVID-19 is also associated with high levels of myriad cytokines and chemokines, their role as pro-NET factors should also be considered.

NETs and SARS-CoV-2 pathology

NETs have direct cytotoxic effects against epithelial and endothelial cells. Indeed, one study demonstrated that SARS-CoV-2-associated NETs induce pulmonary epithelial cell death, which results in alveolar damage and fibrosis [31]. NETs likely also potentiate microvascular thrombosis in COVID-19, as multiple autopsy studies have revealed NET-containing microthrombi and neutrophil-platelet infiltration in the microvasculature of the lung, kidney, and heart [31,37–39]. While not well investigated, disproportionate NET formation may also lead to pathogenic autoantibody production that likely contributes to further local or systemic damage. In summary, clinical, pathological, and molecular evidence support the presence of NETs in COVID-19. SARS-CoV-2 can directly and indirectly induce NET formation, which contributes to COVID-19 pathology.

Complement: helpful early in COVID-19, detrimental late

Data from clinical, pathological, and molecular studies suggest an important role for complement activation in the development of severe COVID-19 manifestations. Yet, it is also clear that complement is critical for protective responses early in COVID-19.

Complement in immunity and disease

The complement system is composed of over 40 serine proteases, inhibitors, and receptors initiated by three pathways (classical, lectin, and alternative). Each pathway is uniquely triggered to drive a proteolytic cascade, which generates a potent, highly regulated, innate immune response [40]. Its activation induces: 1) membrane perturbation mediated by C4b- and C3b-facilitated opsonization and phagocytosis as well as a lytic process through the membrane attack complex (**MAC and C5b-9**), and 2) the generation of a proinflammatory milieu largely mediated through the anaphylatoxins, C3a and C5a. Using these two phenomena, the complement system also assists in the clearance of apoptotic material and cellular debris. Additionally, intracellular complement activation enables cells to modulate metabolic pathways and thereby regulate immune responsiveness [41].

These same pathways though are utilized in several complement-mediated diseases. In systemic lupus erythematosus and related autoimmune diseases, immune complexes generated by autoantibodies drive type II and III hypersensitivity reactions, driving classical pathway activation to initiate destructive inflammatory responses. In age-related macular degeneration (**AMD**) and atypical hemolytic uremic syndrome (**aHUS**), loss-of-function (commonly haploinsufficiency) of complement regulatory proteins or gain-of-function in complement-activating components promote excessive alternative pathway engagement, leading to retinal damage in AMD and microthrombi featuring endothelial injury in aHUS [42,43].

Complement activation is likely required for controlling early COVID-19

Complement has a well-established role in immunity against viruses. Initiated by natural and cross-reacting antibodies and lectins, viruses trigger complement action to drive the opsonization of viruses and virus-infected cells, generation of an antiviral inflammatory state, and boost virus-specific immune responses [44].

Indeed, several lines of data support that SARS-CoV-2 can directly activate complement. *In vitro*, SARS-CoV-2 spike proteins 1 and 2 expressed on the human cell line PIGAnull TF1 primarily drives the activation of the alternative pathway through heparan sulfate [45], and in a preprint, the N protein activated the mannan-binding lectin-associated serine protease (**MASP-2**) [46]. Furthermore, virus infection appears to drive the early activation of complement *in vivo*, as in a murine model of SARS-CoV, infected mice generate C3 activation products (C3a, C3b, iC3b, C3c, and C3d) in the lung within 24 h [47]. While numerous reports have observed elevated complement activation product levels in severe COVID-19 patients (as discussed later), only one report assessed complement component levels in early COVID-19 infection. Here, low complement C3 was found in 57% of day 1 samples in COVID-19 patients who eventually developed nonserious pneumonia [48]. This may be an underestimation of complement activation though, as C3 is an acute phase reactant [49]. Thus, normal C3 levels may also represent elevated complement activation status due to the elevated production of C3 in inflammatory states.

To counteract complement activation, viruses have developed evasion tactics. While these have yet to be observed for coronaviruses, other viruses have well-described mechanisms (reviewed in Ref. [50]). For example, poxviruses express a protein with similar structure and function to complement regulatory proteins, while flaviviruses neutralize complement regulators by binding to them.

While our understanding of the role of complement in early COVID-19 remains in its infancy, we do speculate that complement, along with other immune pathways such as interferons and neutrophil activation, serve to drive important antivirus responses. If compromised, this can lead to inadequate control of SARS-CoV-2, which leads to severe manifestations of COVID-19.

Complement contributes to immunopathology observed in severe COVID-19

Initial data that support a prominent role of complement in severe COVID-19 came from clinicopathological studies. Jeffrey Laurence's group identified complement deposition (specifically C5b-9, C4d, and MASP-2), in the skin of deceased COVID-19 patients with retiform and purpuric lesions and the lung of those with septal capillary injury [25]. Notably, they also found the C4d and C5b-9 colocalized with SARS-CoV-2 S protein in these organs [25]. Similarly, mannose-binding lectins (**MBL**), C4, C3, and C3b-9 were observed in alveolar epithelial cells and alveolar exudate in deceased patients [46]. Furthermore, C5b-9 deposition was also observed in the apical brush border of tubular epithelial cells in the kidney [51].

Additional histopathological features with similarities to other complement-mediated diseases indicate that complement activation is a central player in severe COVID-19. Endothelial cell abnormalities, such as cellular swelling with foamy degeneration in the setting of a thrombotic microangiopathy (**TMA**), have been observed in numerous organs [8,52–56], consistent with C5b-9 mediated injury and a hypercoagulable state [57]. In the lungs of those with COVID-19-associated ARDS, septal microangiopathy was observed characterized by endothelial cell injury, mural fibrin deposition, and variable intraluminal thrombus formation [8,58,59]. Consistent with these pathological observations, C3-deficient mice infected with SARS-CoV demonstrated less lung inflammation and injury and weight loss despite having similar viral loads as compared to wild-type mice [47].

Both proteomic [46,60–64] and transcriptomic [62,65] studies of blood and lung samples from severe COVID-19 confirm the notion that complement activation is an immune signature characteristic of severe disease. The earliest data were observed in Chinese COVID-19 patients, as elevated serum C5a was uniquely noted in those with severe respiratory distress or hypoxia on room air as compared to those with mild symptoms or healthy controls [46]. C5a was confirmed as a potential biomarker of severe disease in a longitudinal analysis of a French cohort, and both anti-C5aR1-treated or C5aR1-knockout mice had attenuated lung injury following C5a instillation into the lungs [66]. MBL was

associated with plasma D-dimer concentration in a Swedish cohort of critically ill COVID-19 patients [67]. Using the Albany Medical Center (New York) cohort, plasma target metabolomics identified complement activation and its regulation as two of the top six GO (gene ontogeny) pathways unique to hospitalized COVID-19 patients who require ICU admission as compared to those on the medical floor [64]. Data from the Columbia University Irving Medical Center/New York-Presbyterian Hospital cohort confirmed these observations as hospitalized patients with COVID-19 exhibited elevated serum levels of factor H and I along with C5, as assessed by mass spectroscopy, as compared to healthy controls [60]. Using immunoassays in the Norwegian cohort, elevated plasma sC5b-9, C5a, C3b/iC3b/C3c, alternative pathway C3 convertase (C3bBbP), and C4d were observed in majority of COVID-19 patients in respiratory failure and were higher as compared to those that were not [61]. Similar observations were made in the lungs at the transcriptomic level, as lung biopsy samples from COVID-19 patients showed a pronounced complement signature (including C3 transcripts) following GO pathway analysis as compared to healthy controls [65]. They confirmed this observation using *in vitro* SARS-CoV-2-infected primary bronchial epithelial cells or a type II human pneumocyte cell line (A549) with or without ACE2 overexpression, which demonstrates a similar complement signature, which was not observed in influenza A- or respiratory syncytial virus-infected cells [65].

Collectively, these data provide substantial evidence that complement activation occurs in COVID-19, with emerging data that this positively correlates with disease severity.

Interplay between NETs and complement

As discussed above, NETs are webs of extracellular chromatin decorated with factors derived from neutrophil cytoplasm, granules, and mitochondria. NETs restrain invading microbes through both immobilization and killing, but when released intravascularly they are an important nidus for thromboinflammation. For example, NETs activate platelets [68], capture red blood cells [68], and potentiate both intrinsic [13] and extrinsic pathways [69] of coagulation.

Prior knowledge regarding the interplay between NETs and complement

The communication between NETs and complement is bidirectional and, if not tightly regulated, has potential to form a self-amplifying loop. C5a recruits and then primes neutrophils for NETosis through the upregulation of various immune receptors, including TLRs and complement receptors such as CR1 and CR3 [70,71]. Pathogens opsonized with C3b and iC3b may then trigger NETosis through the engagement of neutrophils CR1 and CR3, respectively [72]. Furthermore, once NETs have been released into the extracellular space, complement components—and perhaps particularly C1q—stabilize their structure and prevent clearance by DNase I [73].

Emphasizing the bidirectional nature of the relationship, neutrophils express Factor B, C3, and properdin, the combination of which allows for the production and stabilization of the C3-convertase on the neutrophil surface [74] and also on NETs themselves [75]. At the same time, myeloperoxidase and serine proteases such as cathepsin G and proteinase 3 bind to and activate properdin with the potential to further potentiate complement activation on NETs [76]. The end result is NETs as a fertile platform for the generation of anaphylatoxins C3a and C5a [73], which can lead to further immune system activation.

NETs, complement, and thrombotic microangiopathy

Prior to COVID-19, circulating NET remnants were already known to track closely with the activity of various **TMAs,** including thrombotic thrombocytopenic purpura [77,78], hemolytic uremic syndrome [79,80], transplant-associated TMA [78,81], and likely catastrophic antiphospholipid syndrome [82]. At the same time, these TMAs are also clearly complementopathies as evidenced by the utility of inhibitors of C5 cleavage as effective therapies [83]. One interesting study contrasted transplant-associated TMA with another complication of stem-cell transplant, graft versus host disease (**GVHD**) [84]. While markers of endothelial damage/activation such as thrombomodulin were elevated in both situations, only transplant-associated TMA was associated with elevations in soluble C5b-9, NET

remnants, including myeloperoxidase-DNA complexes and markers of coagulation pathways such as thrombin-antithrombin complexes [84].

The potential for cross talk between complement and NETs has recently been evaluated in COVID-19. Specifically, Skendros and colleagues found that C3 inhibitor compstatin Cp40 disrupted tissue factor expression by COVID-19 neutrophils, while C5a receptor blockade attenuated NETosis triggered by COVID-19 platelet-rich plasma [85]. The group also found that COVID-19 NETs are decorated with particularly high levels of tissue factor and through this and likely other pathways are potent activators of cultured endothelial cells [85]. Taken together, it is not difficult to see how these pathways could conspire to occlude the COVID-19 microvasculature *in vivo*.

Potential approaches to treatment

As we await what will hopefully be definitive antiviral solutions to the current pandemic, anti-neutrophil and anti-complement therapies have potential to help mitigate the severest manifestations of COVID-19.

Medications already used in the treatment of COVID-19

Patients with COVID-19 are increasingly being treated with the combination of heparin-based anticoagulation and dexamethasone. The former may modulate NETs by the neutralization of cytotoxic histones [86] and by the potentiation of NET clearance through DNase I [30]. At the same time, corticosteroids such as dexamethasone can also reduce NET formation *in vivo* [87], most likely through the modulation of the inflammatory mediators that activate neutrophils. Other agents being trialed in COVID-19 such as JAK-STAT inhibitors, anakinra (IL-1 receptor antagonist), and colchicine also have potential to reduce NET release [88–90].

DNases

Recombinant DNase I cleaves the DNA scaffold of NETs and thereby has the potential to relieve intravascular thrombosis and airway obstruction [30]. Applying this logic, a small single-center case series has suggested that nebulized endotracheal dornase alfa (a biosynthetic form of human DNase I) reduced oxygen requirements in all treated patients [91]. Going forward, COVIDornase (NCT04355364) and COVASE (NCT04359654) are two studies evaluating nebulized dornase alfa in prospective randomized controlled trials. While there are not (to our knowledge) any active studies for intravenous administration, one wonders if some of the products might find their way into circulation through the damaged airways of COVID-19 [92].

Treatment by modulation of purinergic signaling with dipyridamole

The activation of surface adenosine receptors suppresses NETosis through cyclic AMP-dependent signaling [93]. Dipyridamole is an inexpensive, FDA-approved drug with a favorable safety profile. Dipyridamole potentiates adenosine receptor signaling by both the inhibition of ectonucleoside reuptake and stabilization of intracellular cyclic AMP. Dipyridamole tempers NET release *in vitro*, which prevents NET-dependent thrombosis in mice [93]. In a small study, dipyridamole suppressed D-dimer levels in patients with COVID-19 [94]. Larger studies are now underway to evaluate clinical outcomes (NCT04391179, NCT04424901) [95].

Treatment with complement inhibitors

C5 has been an early experimental target for severe COVID-19 given that eculizumab is already FDA-approved for several complementopathies. Severe COVID-19 patients treated with C5 cleavage inhibitors (i.e., eculizumab, ravulizumab, and LFG316) have resulted in success in several small case series and reports [96–101], and a small number of patients already on a C5 cleavage inhibitor for PNH or TMA-associated lupus nephritis failed to progress to severe COVID-19 [102–104]. A C5a inhibitor,

vilobelimab (IFX-1), failed to meet the primary endpoint of improved lung function in this small (n = 30) exploratory study, although two secondary outcome measures, pulmonary embolism and renal impairment, were improved in the IFX-1 treatment group [105]. It remains too early to tell whether inhibiting C5a, C5b-9, or both is required to suppress severe manifestations of COVID-19, but several clinical trials inhibiting C5 cleavage (eculizumab: NCT04346797, NCT04355494, ravulizumab: NCT04369469, NCT04390464, and zilucoplan: NCT04382755) and the C5a receptor (avdoralimab: NCT0431367 and IFX-1: NCT04333420) are currently ongoing.

Given its central position, another attractive inhibitory target of complement activation is C3. AMY-101 is a compstatin-based C3 inhibitor that prevents the conformational change of C3 required for subsequent cleavage to C3 and C3b [106], which was described earlier to attenuate neutrophil tissue factor expression [85]. A phase II study is now active examining the utility of AMY-101 (NCT04395456), and a total of four patients with severe COVID-19 have been successfully treated with AMY-101 [101,107]. There may be a theoretical advantage to inhibiting C3 rather than C5, as greater inhibition of NET formation, neutrophil and lymphocyte recovery, and more rapid LDH decline [101].

Additional targets, including the C1 esterase inhibitor (conestat alfa, NCT04414631 and rocunest, NCT04530136) [108] and MASP-2 [109] have also been tried in pilot populations with success.

Summary

In summary, NET-releasing neutrophils and complement appear to play central roles in the immunopathogenesis of COVID-19. This review has presented various points of cross talk between NETs and complement in SARS-CoV-2 infection. These intermingled pathways conspire to promote a thromboinflammatory storm in some individuals with severe COVID-19. Therefore, the development of novel therapeutic strategies that target NET formation and complement activation may help reduce COVID-19 morbidity and mortality.

Practice points

- SARS-CoV-2 displays a complex relationship with the human immune system. The interplay between neutrophils, neutrophil extracellular traps, and complement—important players in innate immunity—contribute to the thromboinflammatory milieu of COVID-19.
- SARS-CoV-2 can directly and indirectly induce NET formation, which contributes to COVID-19 pathology.
- NETs contribute to COVID-19 pathology by:
  - direct cytotoxic effects against epithelial and endothelial cells
  - microthrombi formation and microvascular damage in multiple organs
  - perpetuating pathogenic autoantibody production
- While providing early viral containment, complement activation heightens immunopathology and contributes to COVID-19 severity.
- The cross-talk between NETs and complement are key drivers of COVID-19 thrombotic microangiopathy.
- Anti-neutrophil and anti-complement therapies have potential to mitigate the severest manifestations of COVID-19.

Research agenda

- Deep dive into the mechanisms that govern the tripartite immunopathogenesis of COVID-19 and their downstream and long-term sequela.
- Identify clinically actionable biomarkers for NETs and complement pathway activation thus enables precision management.
- Evaluate therapeutics to target NETs and complement in COVID-19.

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