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Review Article

Activated neutrophils in the initiation and progression of COVID-19: hyperinflammation and immunothrombosis in COVID-19

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Abstract: Coronavirus disease 2019 (COVID-19) is a pandemic respiratory disease caused by a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). COVID-19 is typically associated with fever and influenza-like symptoms in its early stages. Severe cases progress to acute respiratory distress syndrome/acute lung injury (ARDS/ALI), multiple organ damage, and even death. Until now, there has been a lack of specific and definitive treatment for COVID-19, which further challenges the situation. Previous clinical and laboratory data showed that neutrophils were significantly decreased in patients who died from COVID-19 in the early stages of disease; when patients were admitted to the hospital the number of neutrophils increased dramatically from 7 to 14 days after admission, which is correlated to myocardial and liver injury, thromboembolic complications, and poor prognosis. Autopsy findings revealed abundant neutrophil infiltration in the pulmonary capillaries and exudation into the alveolar cavity. Therefore, we speculate that neutrophils may play an important role in the initiation and progression of COVID-19. In this review, the relationship among the dynamic changes in neutrophils, cytokine storms, and the release of neutrophil extracellular traps (NETs) with the progression of COVID-19 was elucidated in detail. With a better understanding of the pathogenic mechanisms this can lead to improved clinical applications which are identified and discussed in this review.

Keywords: COVID-19, neutrophil, cytokine storm, neutrophil extracellular trap, immunothrombosis

Introduction

COVID-19 infection from the SARS-COV-2, is related to a worldwide pandemic. However, due to the lack of specific therapeutic drugs for the novel coronavirus, the situation appears grim. Recent research has demonstrated that SARS-COV2 can directly enter the cell through binding to cell surface angiotensin-converting enzyme 2 (ACE2) receptors, which are highly expressed in type II alveolar epithelial cells (AT2) and endothelial cells (ECs) [1]. Infected cells can initiate an innate immune response to SARS-COV-2. Neutrophils are the most abundant circulating leukocytes and the main components of immune cells, and are the first line of recruitment to injury site [2]. Recently, studies have indicated that a sharp increase in neutrophils in the peripheral blood shows a significant negative correlation with cardiac injury, liver injury, thromboembolic complications, poor prognosis, and even death [3-8]. The neutrophil-to-lymphocyte ratio (NLR) was identified as an independent risk factor for COVID-19 [9]. Autopsy findings have also shown abundant neutrophil infiltration in pulmonary capillaries and exudate into the alveolar cavity [10], as well as inflammatory microvascular thrombi containing NETs in the lung, kidney, and heart; which were suspected to cause multiorgan failure and high mortality in COVID-19 [11]. Therefore, we reviewed the emerging role of neutrophils in the initiation and progression of COVID-19 to promote efforts to identify potential targets for treatment.

Dynamic changes in neutrophils in COVID-19

Although the main manifestation of COVID-19 is respiratory infection accompanied by fever, sore throat, and muscle soreness, COVID-19-associated pneumonia can develop after a few

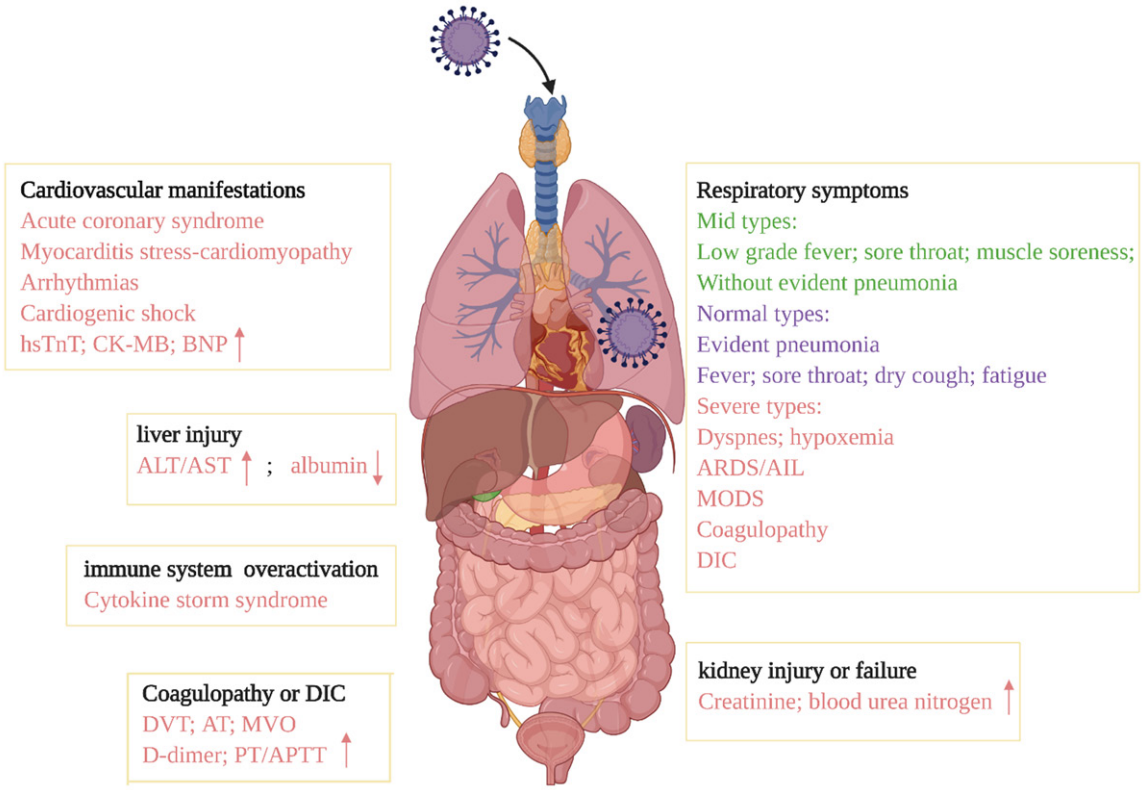

Figure 1. The clinical symptoms, manifestation and hematological findings of COVID-19. Although the main manifestation of COVID-19 caused by SARS-COV2 is a respiratory infection, accompanied by low grade fever, sore throat, and muscle soreness, COVID-19-associated pneumonia can develop after a few days, and severely ill patients can progress to ARDS/AIL accompanied by multiple organ dysfunction syndromes (MODS) including cardiovascular, kidney, liver, hematopoietic and immune system dysfunction, with severe thromboembolic complications. hsTnT, hypersensitive troponin; CK-MB, creatine kinase isoenzyme MB; BNP, brain natriuretic peptide; ALT, glutamic pyruvic transaminase; AST, glutamic aspartate aminotransferase; PT, prothrombin time; APTT, activated partial thromboplastin time; DVT, deep vein thrombosis; AT, arterial thrombosis; MVO, microvascular obstruction; DIC, diffusive intravascular coagulation.

days, and severely ill patients can progress to ARDS/ALI [12]. However, researchers have found that severe cases of COVID-19, can result in significant multiple organ dysfunction syndromes (MODS), including mainly respiratory, cardiovascular, gastrointestinal, nervous, hematopoietic and immune systems, with severe thromboembolic complications [13, 14] (**Figure 1**).

Through further analysis of clinical and laboratory data of patients with COVID-19, the number of neutrophils was found to be significantly higher in the most severe cases or non-survivors than in mild cases or survivors [7, 15]. In addition, polymorphonuclear leukocytes (PMNs), mainly PMN3, are hyporeactive in mild cases; however, neutrophils are hyperactivated in severe COVID-19 [11]. Approximately 7 to 14 days after the onset of initial symptoms, the clinical manifestations of COVID-19 in patients were more prominent, and the number of neutrophils, inflammatory mediators, and cytokines such as IL-1β, IL-6, and TNF-α increased significantly. Inflammatory indexes, including procalcitonin (PCT), lactic dehydrogenase (LDH), C-reactive protein (CRP), and ferritin, increased sharply [16]. Wu et al. found that increased neutrophils were negatively related to the progression of COVID-19-associated pneumonia in ARDS, with increased mortality, and poor prognosis [12]. In patients with complications of myocardial injury or liver injury, researchers have found that the increase in the number of neutrophils was correlated to markers of tissue injury, such as hypersensitive troponin (hsTnT) [3], glutamic pyruvic transaminase (ALT) and glutamic aspartate aminotransferase (AST) [17]. Additionally, in severely ill patients with myocardial injury or severe infec-

tion, coagulation disorders are more common. Therefore, we can infer that neutrophils play an important role in the initiation and progression of COVID-19 and we review the possible pathogenesis to explore the potential clinical treatment of COVID-19.

Neutrophils and ACE2

SARS-COV-2 enters the cell mainly through binding to ACE2 cell surface receptors and this reduces the cell surface ACE2 expression after infection [1]. ACE2 can inhibit neutrophil infiltration and pulmonary inflammation by reducing the activity of the signal transduction pathways and activation of transcription 3 (STAT3) to suppress the IL-17 signaling pathway [18]. Consequently, decreased ACE2 levels in infected alveolar epithelial cells further promotes the aggregation of neutrophils. In addition, ACE2 participates in the regulation of blood coagulation in healthy epithelial cells and vascular endothelial cells. The combination of SARS-COV-2 and the ACE2 receptor leads to cell damage, upregulates the expression of fibrinogen and tissue factor (TF), and inhibits the protein C system [19]. In addition, ACE2, an enzyme involved in the cleavage of angiotensin II to angiotensin 1-7 (Ang1-7), and the internalization and exfoliation of ACE2, results in the inactivation of the ACE2/Ang 1-7/Mas axis [20], promotes vasoconstriction and blood flow reduction, and aggravates hypoxemia.

Neutrophil migration and function

Neutrophils play an essential role in the immune system. In the case of infection, pathogen-related molecular patterns (PAMPs), including lipopolysaccharide (LPS), lipoteichoic acid, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein, are released and recognized by the immune system. PAMPs can bind to, and be sensed by, a variety of pathogen recognition receptors (PRRs) and recruit neutrophils to the injury site for the first time. At the same time, the injured tissue can also release a series of damage-associated molecular patterns (DAMPs), promoting the proinflammatory response and driving neutrophil recruitment into injury sites [21, 22].

Studies have shown that the recruitment of neutrophils to the lung is a critical factor in the pathogenesis of ARDS/ALI [23]. Using RNA-Seq and high-resolution mass spectrometry, Overmyer and his colleagues found that neutrophil activation is closely related to COVID-19 status and severity [24]. In fact, one of the most recent studies has found that mature and activated neutrophil clusters (Neut1 and Neut2) become dominant in patients with COVID-19 ARDS, whereas neutrophils in patients with non-COVID ARDS (caused by bacterial pneumonia) display a more immature phenotypes with enrichment of Neut4 [25]. In addition, some studies have demonstrated the upregulation of chemokines and neutrophils in lung tissue and bronchoalveolar lavage fluid (BALF) of COVID-19 patients, which supports an immunopathological role for neutrophils [10, 26]. Circulating neutrophils activate and change their cytoskeletal structure with retention in the pulmonary capillary bed. Then, neutrophils pass through the postcapillary venule endothelial cells, through the lung interstitial region and epithelial cells, into the alveoli. Neutrophils migrate into the alveolar cavity, inducing epithelial injury and dysfunction, contributing to coagulation activation and fibrin deposition, macrophage activation, interstitial and alveolar edema, and exacerbating tissue dysfunction and the release of cytotoxic mediators. Damaged epithelium and endothelium further promote the development of alveolar edema and hypoxemia and aggravate the proinflammatory state. In addition, the release of neutrophil-derived proteases, and chemokines, can further promote neutrophil recruitment. Available studies indicate that cathepsin C inhibitors and CXCR2 antagonists could be potential therapeutic targets in COVID-19 [27, 28]. In animal models, neutrophil depletion, inhibition of critical chemokines and signaling molecules, or accelerated apoptosis were seen to shorten the lifespan of neutrophils which can improve oxygenation, suppress inflammation, or relieve inflammation [29]. However, the reverse migration of neutrophils may also promote the spread of pathogens, leading to SARS-COV-2 spreading [30].

Cytokine storm

Cytokine storms, also known as inflammatory storms, refer to the phenomenon where the immune system is over activated when the body is infected with microorganisms or other severe stimuli, resulting in the rapid production of a variety of cytokines. Inflammation caused

by cytokine storms begins locally and spreads to the whole body through the circulation, resulting in a variety of diseases such as ARDS, sepsis, acute pancreatitis, and other ailments, and can even be life threatening [31-33]. For some COVID-19 patients who die due to severe hypoxia or MODS in the end-stage, cytokine storms are a potential risk factor for exacerbation. Studies have indicated that the plasma levels of IL-1β, IL-2, IL-6, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), IFN-γ inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), Myo-inositol-1-phosphate (MIP), and tumor necrosis factor-alpha (TNF-α) increased significantly in patients with severe COVID-19 and that these molecules peaked after respiratory failure [16, 34]. Out-of-control cytokine storms will further exacerbate inflammation, leading to alveolar structure damage and endothelial dysfunction. Disrupted alveolar structure and the endothelial barrier worsen hypoxia and eventually progress to life-threatening ARDS. Previous studies have shown that neutrophils also express NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) and are absent in melanoma 2 (AIM2) inflammatory components, which participate in the release of IL-1β. In contrast, the release of inflammatory factors can further promote neutrophil activation [35]. Additionally, neutrophils releasing neutrophil extracellular traps (NETs) and the cytoplasmic components of neutrophils are vital factors in maintaining IL-17-driven neutropenia and NET formation [36].

NETs and COVID-19

Neutrophils, an essential part of innate immunity, play a crucial role in mediating the inflammatory response. After virus infection, neutrophils can migrate along the vessel wall, bind to endothelial selectin/adhesion molecules, and cross the endothelial-epithelial cell layer into the alveoli [37]. In addition, neutrophils can release a reticular structure with double-stranded DNA containing a variety of bactericidal proteins, named NETs, which can effectively eliminate pathogenic microorganisms and inhibit the spread [38]. With the progress of research, it has been found that excessive production of NETs can destroy the endothelial/epithelial cell barrier and they are closely related to myocardial infarction (MI), deep vein thrombosis (DVT), and other thrombosis events such as thrombotic microangiopathy (TMA) and are disseminated in intravascular coagulation (DIC) [39].

Previous studies have found that NETs are involved in the process of lung inflammation caused by a variety of viral infections. Influenza virus A can stimulate extensive NET formation in the alveoli, resulting in airway obstruction in the bronchioles, extending to endothelial damage, and thus disrupting gas exchange [40]. Respiratory syncytial virus (RSV) can also promote the formation of NETs [41]. Moreover, Zuo and his colleagues found excessive neutrophils and neutrophil extracellular traps in the serum of patients with severe COVID-19, which was negatively related to respiratory failure of COVID-19 [6]. In addition, in the autopsy results of patients who died from COVID-19, a large amount of neutrophil infiltration was found in the alveolar capillaries with abundant NETs in the inflammatory microthrombi that were present in the lung, kidney and heart [11, 42], linking multiorgan failure and systemic hypercoagulability to COVID-19. To date, although there is no direct evidence to prove that SARS-COV-2 can directly induce NET production, as the levels of NET markers including cell-free DNA (cf-DNA), myeloperoxidase-deoxyribonucleic acid (MPO-DNA) complex and citrullination of histone H3 (cit-H3), are closely related to the progression and poor prognosis of COVID-19, we can reasonably infer that SARS-COV-2 can promote NET production in COVID-19. However, the mechanism by which NETs promote the progression of COVID-19 still requires further exploration.

Interaction between NETs and epithelial/endothelial cells

NETs interact with epithelial/endothelial cells through the binding of neutrophil β2-integrin with epithelial ligands [40]. Hypoxia can induce hypoxia-inducible factor 1 (HIF-1) expression, and HIF-1 overexpression can help neutrophils adhere to epithelial/endothelial cells by promoting the expression of β2-integrin in respiratory diseases [43]. NETs can also induce G protein-coupled receptor (GPCR)-mediated signal-activated myosin light-chain kinase (MLCK)-dependent actin contraction, dampening proteins at the tight junction and adhesion junction of the apical epithelium/endothelium,

which contributes to epithelial/endothelial barrier function disruption [40, 44]. In addition, proteases, including neutrophil elastase (NE), cit-H3, and metalloproteases (MMPs), distributed on NETs can enhance the permeability of the epithelial/endothelial barrier, while MMP9 expressed on damaged endothelial cells can further promote distal neutrophil aggregation and NET production [45].

Exudation occurs mainly in the microvasculature of the injured tissues/organs, as confirmed by autophagy, which indicates that abundant neutrophil infiltration in pulmonary capillaries, acute endotheliitis and extravasation of neutrophils into the alveolar space [46] facilitates the progression of hypoxia and systematic capillary leakage. The interaction between endothelial cells and NETs during exudation, including homotypic interactions, is followed by the interaction between platelet-endothelial cell adhesion molecule-1 (CD31) [47, 48]. Systemic inflammatory stimuli induce circulating neutrophils to switch to the proinflammatory phenotype and NETosis, increase the secretion of proinflammatory mediators and cytokines, activate inflammatory signals, such as angiopoietin-2 (Ang2)/angiopoietin–tie (Tie2) signals [49], and express adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) [50], further promoting neutrophil/NET migration and adhesion to distant organs. Therefore, the interaction between NETs and epithelial/endothelial cells plays an essential role in the pathogenesis of COVID-19.

NETs and thrombotic complications in COVID-19

To date, many studies have confirmed that thrombotic complications are suspected to contribute to a high mortality in COVID-19 [34, 51]. In recent years, a significant amount of research has shown that NETs can promote the formation of thrombi. In VTE, rolling neutrophils binding with endothelial P-selectin glycoprotein ligand-1 (PSGL-1) and chemokine receptor 2 (CXCR2) can promote the expression of β2 integrin and von Willebrand factor (VWF), contributing to the firm adhesion of neutrophils to endothelial cells and NET formation [52], which binds tightly to the blood vessel wall, preventing blood flow and further promoting endothelial injury [53]. Recent reports have illustrated that massive NETs are enriched in serum and microvascular thrombi, present in the lung, kidney, and heart of severely ill COVID-19 patients [6, 11]. In addition, COVID-19 PRP-induced NETs can activate endothelial cells to express adhesion molecules and tissue factors [50].

Furthermore, NETs can also provide a catalytic surface for circulating procoagulant components such as platelets (PLTs), monocytes, red blood cells (RBCs), microparticles (MPs) and soluble coagulation factors to promote blood coagulation [45, 54]. In addition, the components of NETs, including DNA, can promote blood coagulation through the contact activation pathway [55]. While histones can promote coagulation through a variety of complex pathways. Histones have been proven to be able to directly promote the production of thrombin in platelet-rich plasma (PRP) [56] but they can also promote the release of platelet polyP [57], phosphatidylserine (PS) exposure and Factor V activation, enhancing the formation of prothrombin complexes [55, 58]. SARS-CoV-2 binding to the endothelial ACE2 receptors can induce endothelial cell activation/injury [1], further increasing the risk of VTE. In addition, the use of hormones and immunoglobulins in severely ill patients may result in increased blood viscosity. Mechanical ventilation, central venous catheterization, and surgery may also aggravate vascular endothelial injury and further promote the occurrence of thrombotic events.

Therefore, we can infer that NETs play an important role in thrombotic complications in COVID-19. Additionally, inflammatory microvascular thrombi can eventually exacerbate pulmonary arterial hypertension (PAH), respiratory failure and MODS by preventing blood flow.

Potential targets in COVID-19

Due to the lack of specific antiviral drugs, the effect of antiviral therapy alone in COVID-19 is minimal. Early clinical trials have shown that lopinavir-ritonavir [59] was not effective in the treatment of patients who are severely ill with COVID-19. The efficacy and toxicity of ribavirin in other coronavirus-associated diseases suggest that the efficacy of treatment with ribavirin for COVID-19 may be limited [60]. The drugs dipyridamole [61] and chloroquine/hydroxy-

Table 1. Potential therapeutics to modulate neutrophil activation and NETs formation

Agent	Target or function	Effects on NETs	Clinical indication
DNase I	DNA	DNA Degradation of NETs	Cystic fibrosis, SLE
Sivelestat, AZD9668	NE	Inactivation of NE in NETs	ARDS, Bronchiectasis
PF-1355	MPO	Inactivation of MPO	ALI
LMWH	Histones	Inactivation of histones	Anticoagulation
Colchicine	Destabilization of actin cytoskeleton	Degradation of NETs	Gout
NAC	ROS scavenger	Reduced NET formation	SLE, liver injury
Anti-TNF antibodies	TNF	Reduced NET formation	RA, IBD
Anti-IL-6 antibodies	IL-6	Reduced NET formation	RA, uveitis, optic neuromyelitis
Anti-IL-17 antibodies	IL-17	Reduced NET formation	RA
Avacopan, eculizumab	C5a chemotactic receptor inhibitors	Reduced NET formation	ANCA-associated vasculitis

LMWH, low molecular heparin; NE, elastase; ANCA, anti-neutrophil cytoplasmic autoantibodies; C5a, anaphylatoxin released from the cleavage of complement C5; NAC, N-acetylcysteine; NET, neutrophil extracellular trap; RA, rheumatoid arthritis; ROS, reactive oxygen species; SLE, systemic lupus erythematosus.

chloroquine [6, 50] inhibited neutrophil activation and NET production, increased virus clearance, improved imaging results, and delayed the development of COVID-19. Therefore, targeting neutrophils and regulating NET production can be a potential target for COVID-19 treatment.

Anti-inflammatory agents

Recent research has demonstrated the benefit of the use of corticosteroids in COVID-19-related ARDS and prednisone treatment can reduce the mortality of COVID-19 [15]. However, the latest research has shown that pathological neutrophils priming and NET production are not modified by corticosteroids treatment, these data suggest that ancillary therapies (eg. NETs-targeted therapies) may yield significant benefits to corticosteroids and provide new ideas for the treatment of COVID-19 ARDS [25]. In addition, intravenous immunoglobulin IgG combined with LMWH was effectively given for 5 days in patients who were severely ill [62].

Immunomodulatory therapies

Neutrophils produce a variety of cytokines through Janus kinases (JAKs); thus, JAK inhibitors may also be emerging drugs for the treatment of COVID-19. The JAKs inhibitors tofacitinib and ruxolitinib have been used in the treatment of various inflammatory diseases, such as RA [63]. In addition, the use of the JAKs inhibitor ruxolitinib has been shown to possibly be effective for clinical improvement in the prognosis of COVID-19 [64]. At the same time, inhibition of tumor necrosis factor (TNF), interleukin-6 and interleukin-17, which are significantly increased in patients with COVID-19 [16], can also reduce the formation of NETs in patients with RA. Anti-tumor necrosis factor monoclonal antibodies have been used to treat RA [65] and inflammatory bowel disease (IBD) [66]. Blocking IL-6 activation has been successfully used in the treatment of a variety of chronic inflammatory diseases, including RA, uveitis, and optic neuromyelitis [67]. In addition, early phase clinical trials indicate a possible clinical benefit of IL-6-modulatory therapies for COVID-19 [68]. Anti-IL-17 antibodies have also shown some efficacy in the treatment of SLE, possibly partly by reducing NET formation [69]. Inhibition of the complement pathway may also be a possible therapeutic strategy. In anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, C5a can activate neutrophils, inducing endothelial injury. Avacopam and eculizumab, selective C5a anaphylatoxin chemotactic receptor 1 inhibitors, can effectively replace high-dose glucocorticoids in the treatment of ANCA-associated vasculitis [70]. Treatment with the anti-C5 monoclonal antibody in an SLE mouse model improved the survival rate of mice [71]. With the application of the complementary inhibitor eculizumab in severely ill COVID-19 patients, the level of CRP decreased, and their prognosis was improved [72].

NETs formation inhibitors

The clinical NET inhibitors are summarized in **Table 1** and are expected to work in the treatment of COVID-19. DNase I has been used in

Table 2. Recommend of antithrombotic drugs

Type of anticoagulant	Agent	Target or function	Clinical indication	Monitoring indexes
Heparin	UFH	Mainly anti-IIa activity	AF, VTE, DIC	APTT, PT, anti-Xa assay
	LMWH	Mainly anti-Xa activity	VTE, ACS	anti-Xa assay
DOAC	Rivaroxaban	Anti-Xa activity	VTE, HIF, AF	
	Apixaban			
	Dabigatran	Anti-IIa activity	VTE, ACS, AF	APTT, CT, TT
VKA	Warfarin	Inhibit the synthesis of II, VII, IX, X	AF, VTE	PT/INR
Thrombolytic drugs	tPA	fibrinolytic	Stroke, MI	fibrinogen
	uPA			
NSAID	Aspirin	Inhibit platelet COX-1and TXA2	VTE, ACS, TIA, stroke et al.	PLT, APTT
P2Y12 Inhibitors	Clopidogrel	Block platelet glycoprotein IIb/IIIa	ACS	
	Tigrillo			
hrAPC		Anti-V and VIII activity	Sepsis, ARDS	PLT, APTT

VKA, vitamin K agonist; UFH, unfractionated heparin; LMWH, low molecular weight heparin; DOAC, direct oral anticoagulant; NSAID, nonsteroidal anti-inflammatory drug; hrAPC, human recombinant activated protein C; COX-1, cyclooxygenase-1; TXA2, thromboxane A2; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; PLT, platelet; CT, clotting time; TT, thrombin time; DIC, disseminated intravascular coagulation; ACS, acute coronary syndromes; AF, arterial fibrillation; VTE, venous thromboembolism; HIT, heparin induced thrombocytopenia; urokinase, uPA; tissue-type plasminogen activator, tPA.

the treatment of cystic fibrosis, and its safety has been confirmed, making it a very feasible option for the treatment of other diseases [73]. In addition, the effectiveness of DNase I treatment was confirmed in an AIL mouse model [74] and systemic lupus erythematosus (SLE) clinical treatment [75]. In addition, current evidence indicates that dornase alfa mayl be well-tolerated by severely ill COVID-19 patients, with reduced oxygen requirements [76]. However, DNase I only destroys the reticular structure of NETs, and it has little effect on histones, elastase, and other proinflammatory components of NETs. Colchicine can also destroy the actin cytoskeleton of NETs and promote their degradation [77]. Neutrophil elastase plays a vital role in tissue damage caused by various neutrophil-mediated diseases and is considered to be a potential target for respiratory diseases [78, 79]. In addition, Taguchi and colleagues found that SARS-CoV-2 activation was mediated by elastase [80]. Although the NE inhibitor sivelestat had no significant effect on improving mortality in patients with ARDS/ALI [81], AZD9668, a neutrophil elastase inhibitor, improved lung function [82]. PF-1355, a selective inhibitor of MPO, can effectively reduce immune-complex-mediated alveolar tissue injury [83]. The reactive oxygen species (ROS) scavenger N-acetylcysteine has been shown to improve acute liver failure and ischemia-reperfusion injury [84]. Moreover, an increasing number of researchers have found that neutrophil-induced oxidative stress in COVID-19 can accelerate tissue damage, affecting RBC membranes and hemoglobin function, which promotes COVID-19 [85, 86].

Antithrombotic therapies

With the high occurrence of thrombotic complications in COVID-19, other common anticoagulation and antiplatelet therapies may be considered (**Table 2**). Autopsy findings indicated that 58% of patients had vein thrombosis and less frequent arterial thromboembolism, such as stroke and MI [87]. However, because fibrin is the dominant component in vein thrombosis, anticoagulation therapy has been the current cornerstone for the prevention and treatment of vein thrombosis. Arterial thrombosis is full of platelets, so antiplatelet therapy is the basis for the treatment of arterial thrombosis. Venous thrombosis accounts for a substantial proportion of COVID-19 thrombotic events, so anticoagulant therapy is recommended as the main treatment.

Unfractionated heparin (UFH) and low molecular weight heparin (LMWH), as the most commonly used anticoagulants, also have potential anti-inflammatory and antiviral effects by blocking selectin, inhibiting bradykinin release and binding to inflammatory factors [88]. LMWH mainly inhibits the coagulation Xa factor, and bleeding risk is lower and anti-inflammatory effects are better than UFH. Considering that UFH may cause fatal thrombocytopenia, severe

COVID-19 may be accompanied by consumptive thrombocytopenia [89], so LMWH is recommended unless the patient has severe renal damage. In severely ill patients with COVID-19, anticoagulation with LMWH can significantly improve the prognosis with D-dimer >3.0 μg/mL compared with UFH [90]. In addition, the vitamin K antagonist warfarin is also commonly used in vein thrombosis anticoagulant therapy, with a high risk of bleeding, and requires monitoring the target international normalized ratio (INR) 2-3. Low-intensity warfarin did not reduce the risk of bleeding; however, low-intensity warfarin increased the risk of occurrence of vein thrombosis by 2.8 times [91]. Therefore, using warfarin in COVID-19 anticoagulant therapy not only needs to be weighed against the risk of thrombosis and bleeding, but it also needs fair use. Our study and other studies have shown that NETs can promote blood coagulation by combining with Xa, IIa, and Ia [45, 54]. At the same time, the new oral anticoagulant (NOAC), unlike warfarin, acts on multiple coagulation factors with a higher risk of bleeding. There is little interaction between NOACs and food or drugs, and when using NOACs physicians do not need to monitor routine blood clotting indicators, which can reduce the decline of drug efficacy or adverse bleeding events caused by improper use of drugs [92]; however, extreme caution should be taken when using it in patients with severe renal damage. Studies have also shown that the thrombolytic drugs urokinase (uPA) and tissue-type plasminogen activator (tPA) can improve oxygenation and ventilation parameters and prevent the progression of ARDS in animal models [93], so thrombolytic therapy should also be considered in COVID-19 treatment. However, the benefits of thrombolytic drugs may be offset by the considerable risk of bleeding, and using nebulation in a way that only increases the local concentrations, is recommended.

Studies have shown that antiplatelet therapy is beneficial to ARDS. In animal models, antiplatelet-derived chemokine antibodies can reduce the incidence of lung injury. In the ALI mouse model, aspirin decreased the level of thromboxane A2 (TXA2) and pulmonary edema, increasing the survival rate. At the same time, taking aspirin prehospital can reduce the mortality of ARDS patients in the ICU [94], probably due to the effects of aspirin, which can induce cyclooxygenase acetylation and convert arachidonic acid into precursor molecules. Furthermore, neutrophils produce lipids through precursor molecules, activate lipoproteins, and inhibit inflammation [94]. In addition, the use of glycoprotein IIb/IIIa blockers such as abciximab, eptifibatide, clopidogrel, and tigrillo in models with endotoxin-induced shock reduced mortality [95, 96]. Moreover, in Japan it was shown that the NE inhibitor sivelestat combined with human recombinant activated protein C (APC) therapy could significantly improve the prognosis of ARDS in clinical trials [97].

Other emerging strategies

Statins are widely used as hypolipidemic drugs; however, a growing body of evidence suggests that statins can also improve endothelial function and inhibit inflammation and thrombogenicity [98]. Because COVID-19 is a disease with an excessive inflammatory response, hypercoagulable tendency and endothelial damage, statins may be used as candidate drugs for the treatment of COVID-19. In addition, recent studies have shown that metformin, as a classic hypoglycemic drug, can also reduce the NLR in patients with diabetes [99]. Metformin has also been seen to reduce neutrophil and macrophage infiltration in premature infants with hypoxia-induced lung injury [100]. COVID-19 patients are often complicated with diabetes, and increased neutrophil count is closely related to a poor prognosis. Thus, we can speculate that metformin may be an emerging drug for the treatment of COVID-19.

Conclusions

As one of the primary immune cells, neutrophils are proven to be one of the critical factors in the pathogenesis of ARDS/AIL. Clinical data have also shown that the continuous increase in neutrophils was negatively related to myocardial and liver injury, thrombus-related complications, poor prognosis, and high mortality of COVID-19. Because neutrophils can eliminate invasive pathogens and protect the lungs, simple knockout of neutrophils is not available for treatment. However, research on the pathogenic mechanism of neutrophils in COVID-19 needs further investigation, but hopefully we can offer potential targets for the treatment of COVID-19 by elucidating the essential function of neutrophils, the correlation between the cytokine storm, the formation of NETs and the progression of the disease (**Figure 2**).

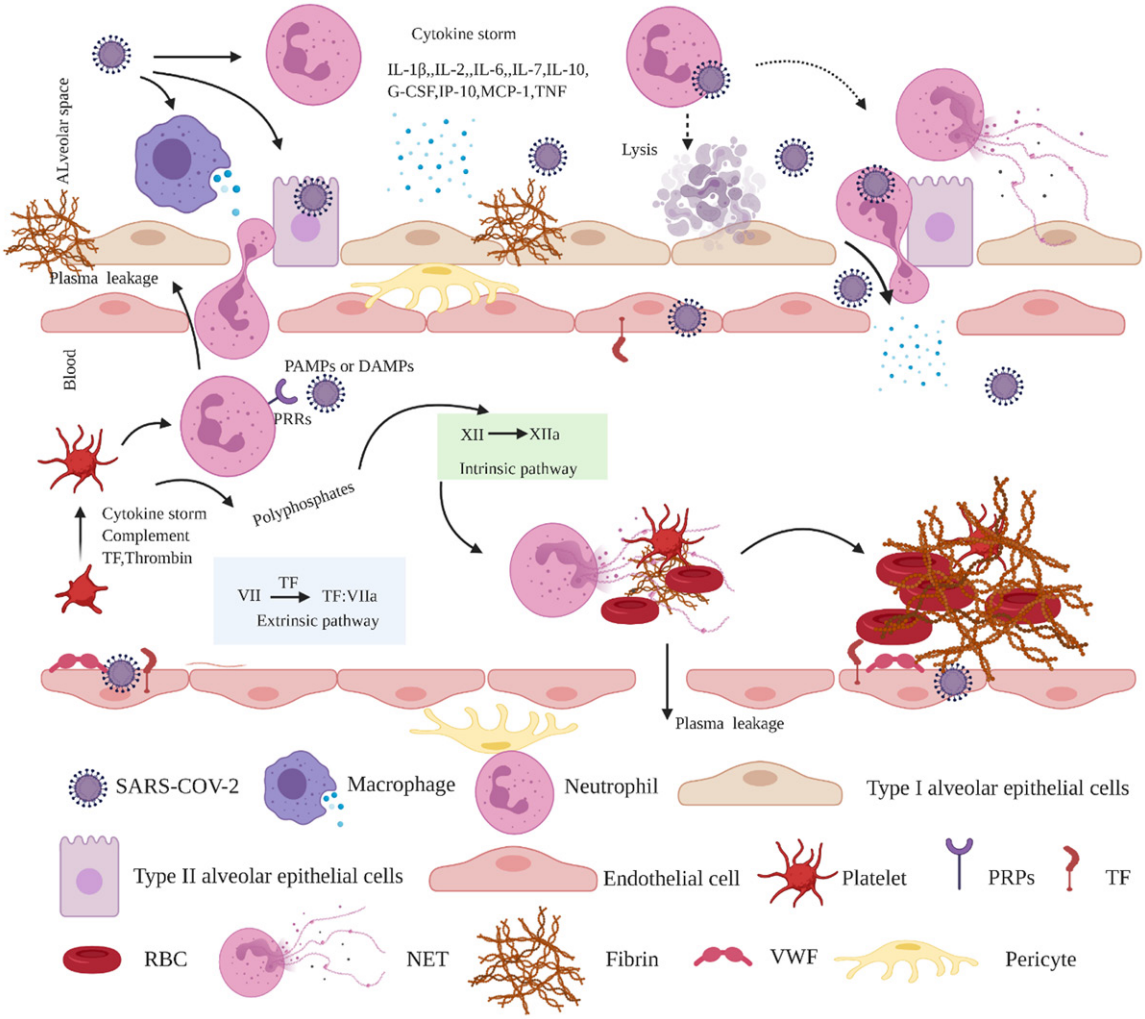

Figure 2. Effect of neutrophils on inflammation and coagulation in COVID-19. Type II alveolar epithelial cells and endothelial cells (ECs) are the primary targets of SARS-COV-2 infection. SARS-COV-2 can directly infect ECs inducing EC activation, and vascular hyperpermeability through inflammatory cytokines, leading to neutrophil activation and recruitment. Second, ECs activation induces a prothrombotic state by down regulating anticoagulant components, the expression of tissue factor (TF), and von Willebrand factor (VWF) and the exposure of collagen to blood. In addition, activated neutrophils release inflammatory cytokines and neutrophil extracellular traps, exacerbating EC damage. Then, activated neutrophils pass through postcapillary venule endothelial cells, through the lung interstitial region and epithelial cells, into the alveoli, contributing to epithelial injury and dysfunction, coagulation activation and fibrin deposition, macrophage activation, and interstitial and alveolar edema, as well as exacerbating tissue dysfunction and the release of cytotoxic mediators. Finally, the reverse migration of neutrophils can also promote the spread of pathogens, leading to spreading of SARS-COV-2.

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Disclosure of conflict of interest

None.

Abbreviations

COVID-19, coronavirus disease 2019; SARS-COV-2, severe acute respiratory syndrome coronavirus 2; ARDS, acute respiratory distress syndrome; ALI, cute lung injury; ACE2, angiotensin-

converting enzyme 2; AT2, type II alveolar epithelial cells; ECs, endothelial cells; NLR, neutrophil-to-lymphocyte ratio; MODS, multiple organ dysfunction syndromes; ICU, intensive care unit; PCT, procalcitonin; LDH, lactic dehydrogenase; CRP, C-reactive protein; hsTnT, hypersensitive troponin; ALT, glutamic pyruvic transaminase; AST, glutamic aspartate aminotransferase; STAT3, signal transducer and activator of transcription 3; TF, tissue factor; Ang1-7, angiotensin 1-7; PAMPs, pathogen-related molecular patterns; LPS, lipopolysaccharide; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; PRRs, pathogen recognition receptors; DAMPs, damage-associated molecular patterns; G-CSF, granulocyte colony-stimulating factor; IP-10, IFN-γ inducible protein 10; MCP-1, monocyte chemoattractant protein-1; MIP, Myo-inositol-1-phosphate; TNF-α, tumor necrosis factor-alpha; NLPR3, NOD-, LRR- and pyrin domain-containing protein 3; AIM2, absent in melanoma 2; NETs, neutrophil extracellular traps; RA, rheumatoid arthritis; TNFR, tumor necrosis factor receptor; MI, myocardial infarction; DVT, deep vein thrombosis; TMA, thrombotic microangiopathy; DIC, disseminated intravascular coagulation; RSV, respiratory syncytial virus; COPD, chronic obstructive pulmonary disease; cf-DNA, cell-free DNA; MPO-DNA, myeloperoxidase-deoxyribonucleic acid; cit-H3, citrullination of histone H3; HIF-1, Hypoxia-inducible factor 1; GPCR, G protein-coupled receptor; MLCK, myosin light-chain kinase; NE, neutrophil elastase; MMPs, metalloproteases; PCVs, postcapillary venules; Ang2, Angiopoietin-2; Tie, angiopoietin–tie; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand-1; CXCR2, chemokine receptor 2; VWF, von Willebrand factor; PLT, platelet; WBC, white blood cells; RBC, red blood cells; MPs, microparticles; PRP, platelet-rich plasm; PS, phosphatidylserine; JAKs, Janus kinases; IBD, Inflammatory Bowel Disease; ANCA, anti-neutrophil cytoplasmic antibodies; SLE, systemic lupus erythematosus; UFH, Unfractionated heparin; LMWH, low molecular weight heparin; INR, international normalized ratio; NOAC, new oral anticoagulant; uPA, urokinase; tPA, tissue-type plasminogen activator; TXA2, thromboxane A2; APC, activated protein C.

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