Review Article

Neutrophil extracellular traps (NETs): the role of inflammation and coagulation in COVID-19

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Abstract: COVID-19 has swept quickly across the world with a worrisome death toll. SARS-CoV-2 infection induces cytokine storm, acute respiratory distress syndrome with progressive lung damage, multiple organ failure, and even death. In this review, we summarize the pathophysiologic mechanism of neutrophil extracellular traps (NETs) and hypoxia in three main phases, focused on lung inflammation and thrombosis. Furthermore, microparticle storm resulted from apoptotic blood cells are central contributors to the generation and propagation of thrombosis. We focus on microthrombi in the early stage and describe in detail combined antithrombotic with fibrinolytic therapies to suppress microthrombi evolving into clinical events of thrombosis. We further discuss pulmonary hypertension causing plasmin, fibrinogen and albumin, globulin extruding into alveolar lumens, which impedes gas exchange and induces severe hypoxia. Hypoxia in turn induces pulmonary hypertension, and amplifies ECs damage in this pathophysiologic process, which forms a positive feedback loop, aggravating disease progression. Understanding the mechanisms paves the way for current treatment of COVID-19 patients.

Keywords: COVID-19, neutrophil extracellular traps, hypoxia, thrombosis, acute respiratory distress syndrome, treatment strategy

Introduction

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical symptoms of some patients include rapid progression from mild symptoms to acute respiratory distress syndrome (ARDS), multiple organ failure (MOF), and even death. Excessive pro-inflammatory cytokines, such as Interleukin (IL)-6, tumor necrosis factor (TNF)-α, and Interleukin (IL)-1β, are described in a cytokine storm [1]. Reports of thrombotic events are frequent and preventive antithrombic therapy in clinical practice is required for hospitalized patients. Previous studies suggested that thrombosis was associated with platelet activation, endothelial cell (EC) dysfunction, inflammation, and the complement system. Recent studies have revealed neutrophil infiltration in the alveoli and neutrophil extracellular traps (NETs) formation in serum in COVID-19 patients, which is predictive of adverse results [2].

The excessive or inappropriate production of NETs occurs in various pathologic processes, including inflammation, thrombosis, ARDS, and MOF. However, the underlying mechanism of NETs that triggers severe thrombotic illness in SARS-CoV-2-infected patients is not yet completely understood.

Progression from virus invasion to systemic hyperinflammation

SARS-CoV-2 infection mainly consists of three phases: the initial phase including viral invasion and minor symptoms; the second phase involving coagulation abnormalities and respiratory symptoms (pulmonary phase); the third phase with progression to systemic thrombosis, hyperinflammatory state, and MOF (systemic hyperinflammatory phase) [3]. NETs are strongly implicated in disease progression and adverse outcome. Recent reports have highlighted the role of NETs in COVID-19 patients...
Therefore, we elucidate the important role of NETs in the three phases.

**Phase I: viral invasion and minor symptoms**

In the first phase, the SARS-CoV-2 invades cells in the mucous membranes and ultimately reaches the lungs by the respiratory tract. Viral Spike (S) protein binds with angiotensin-converting enzyme 2 (ACE2) and is initiated by the transmembrane protease serine 2 (TMPRSS2), leading to virus infection, infiltration and proliferation in the lung parenchyma. Neutrophil elastase (NE), the main component of NETs, results in S protein cleavage and entry into cells directly from the cell surface, increasing viral infectivity [5]. SARS-CoV-2 infects lung tissue and induces necrosis and sloughing of alveolar epithelial cells, which results in inflammatory cell infiltration and pro-inflammatory cytokine release in the lungs [6]. Infiltrated neutrophil and other immune cells consume oxygen, resulting in local consumptive hypoxia. Under this circumstance, hypoxia-induced hypoxia inducible factor-1α (HIF-1α) is necessary for neutrophil survival and macrophage phagocytosis. Neutrophils generate excessive reactive oxygen species (ROS) that aggravate the host immune response [7]. ROS further contribute to the generation of NETs, which entrap virus and prevent their dissemination into the circulation. Macrophages infiltrating in the lung have been shown to clear virus by phagocytosis. The main symptoms manifest as fever, dry cough, myalgias, fatigue and headache at this stage. However, recent studies have revealed that some COVID-19 patients in the early phase prior to admission show elevated D-dimer levels and microthrombi formation (Table 1). This phase involving viral invasion and replication is characterized by minor symptoms and shows an antiviral response mainly triggered by NETs and macrophages.

**Phase II: pulmonary phase**

The second phase is mainly characterized by lung inflammation and injury. NET generation is mainly responsible for the inflammatory response of lung tissues. The components of NETs, extracellular cationic histones, exert cytotoxic activity to facilitate the generation of IL-1β and mediate pro-inflammatory effects by activating toll-like receptor 2 (TLR2), TLR4, and TLR9 [8]. NETs enhance various cytokines production, including IL-1β and TNF-α by extracellular signal-regulated kinase/mitogen-activated protein kinase activation to enhance inflammatory response [9]. Moreover, IL-1β and TNF-α, as the main activators, also induce increased IL-6 levels [10]. Additionally, NETs components can facilitate the inflammatory response by mediating complement activation, regarded as inflammasome activators and danger-associated molecular patterns [11]. NETs can induce tissue damage by extracellular exposure of DNA, granular proteins including myeloperoxidase and histone, leading to apoptosis and fibrotic processes [12, 13]. Recent studies show that SARS-CoV-2 can directly stimulate neutrophils to release NETs dependent on ACE2 and serine protease activity axis, and NETs induce lung epithelial cell death [14]. NETs are involved in an inflammatory response and lung injury.

Hypoxemia, tachypnea, and breathlessness are commonly seen in this phase. Hypoxia upregulates tissue factor (TF) expression in phagocytes to promote thrombosis by activating early growth response-1 transcription factor [15]. Additionally, hypoxia promotes inflammation by increased IL-6 and C-reactive protein (CRP) [16]. Hypoxia-inducible factor (HIF) is expressed on neutrophils and macrophages under hypoxic conditions. Inappropriate activation of HIF induces neutrophil persistence, resulting in delayed inflammation resolution and tissue damage [17]. Hypoxia is critical for determining lung epithelial cell fate and up-regulation of HIF-1α expression inhibits the recovery of damaged epithelial cells.

In COVID-19 patients, extensively abnormal laboratory values involve increased inflammatory markers, including IL-1, IL-6, TNF-α, CRP, ferritin, as well as coagulation abnormalities, consisting of D-dimer, prothrombin time (PT), fibrinogen, fibrinogen degradation products (FDPs), platelet count, von Willebrand factor (vWF), antithrombin (AT), and coagulation factor VIII (FVIII) (Table 2). The characteristic changes of coagulation values, D-dimer and FDP, fibrinogen and platelet count, indicate the activation of coagulation in response to the inflammation and virus infection [18] (Table 1).

**Phase III: systemic hyperinflammatory state**

In the third phase, as the inflammatory responses are further reinforced, more neutrophils recruited into inflamed lung tissue and releas-
### Table 1. Laboratory values and major mechanisms involved in different stages in COVID-19

<table>
<thead>
<tr>
<th>The disease stage</th>
<th>Laboratory parameters</th>
<th>Dominant mechanisms involved</th>
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</thead>
<tbody>
<tr>
<td><strong>Initial phase</strong></td>
<td>D-dimer levels ↑</td>
<td>Virus invasion</td>
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<td></td>
<td></td>
<td>Antiviral response</td>
</tr>
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<td></td>
<td></td>
<td>a. Local consumptive hypoxia induced by infiltrated neutrophil and other immune cells.</td>
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<td>b. ROS release, NETs generation and macrophages infiltrating.</td>
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<tr>
<td></td>
<td></td>
<td><strong>Antiviral response</strong></td>
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<tr>
<td></td>
<td></td>
<td>Lung inflammation and injury</td>
</tr>
<tr>
<td><strong>Pulmonary phase</strong></td>
<td>Interleukin-1 ↑</td>
<td>Lung inflammation and injury</td>
</tr>
<tr>
<td></td>
<td>Interleukin-6 ↑</td>
<td>Lung inflammation and injury</td>
</tr>
<tr>
<td></td>
<td>TNF-α ↑</td>
<td>Lung inflammation and injury</td>
</tr>
<tr>
<td></td>
<td>C reactive protein ↑</td>
<td>The role of hypoxia in the pulmonary phase</td>
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<tr>
<td></td>
<td>Ferritin ↑</td>
<td>The role of hypoxia in the pulmonary phase</td>
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<tr>
<td></td>
<td>Platelet count ↓</td>
<td>Lung inflammation and injury</td>
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<tr>
<td></td>
<td>vWF ↑</td>
<td>Lung inflammation and injury</td>
</tr>
<tr>
<td></td>
<td>D-dimer ↑</td>
<td>Lung inflammation and injury</td>
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<tr>
<td></td>
<td>Factor VIII ↑</td>
<td>Lung inflammation and injury</td>
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<tr>
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<td>Hyperinflammatory state</td>
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<td>Pulmonary pathophysiology</td>
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<td></td>
<td>Pulmonary hypertension, ECs damage and inflammation collectively are involved in the pathophysiology of lung.</td>
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<td>Thrombosis</td>
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<td>NETs are involved in ARDS and MOF.</td>
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<tr>
<td><strong>Systemic hyperinflammatory phase</strong></td>
<td>Lymphocyte count ↓</td>
<td>Hyperinflammatory state</td>
</tr>
<tr>
<td></td>
<td>D-dimers ↑</td>
<td>NETs and pro-inflammatory substances released by macrophages constitutes a positive feedback loop, forming a cytokine storm.</td>
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<td></td>
<td>Soluble TM ↑</td>
<td>Pulmonary pathophysiology</td>
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<td></td>
<td>Soluble P-selectin ↑</td>
<td>Pulmonary hypertension, ECs damage and inflammation collectively are involved in the pathophysiology of lung.</td>
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<td>Soluble CD40 ligand ↑</td>
<td>Thrombosis</td>
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</tbody>
</table>

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; TMPRSS2: The transmembrane protease serine 2; ACE2: Angiotensin-converting enzyme 2; ROS: Reactive oxygen species; NETs: Neutrophil extracellular traps; TNF-α: Tumor necrosis factor-α; FDPs: Fibrinogen degradation products; vWF: von Willebrand factor; TF: Tissue factor; PS: Phosphatidylserine; ECs: Endothelial cells; ADAMTS13: A disintegrin and metalloproteinase with a thrombospondin motif repeats 13; ARDS: Acute respiratory distress syndrome; MOF: Multiple organ failure; COVID-19: Coronavirus disease 2019.
## Table 2. COVID-19-associated coagulation

<table>
<thead>
<tr>
<th>Value</th>
<th>Direction of change</th>
<th>Comparator (case versus control)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer</td>
<td>↑</td>
<td>ICU versus non-ICU</td>
<td>Patients with high D-dimer and high CRP have the greatest risk of adverse outcomes [49, 50].</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>↑</td>
<td>COVID-19 versus healthy control</td>
<td>The severity of COVID-19 was found significantly associated with significant increase of neutrophil to lymphocyte ratio, ferritin, fibrinogen [51].</td>
</tr>
<tr>
<td>TAT</td>
<td>↑</td>
<td>COVID-19 versus healthy control</td>
<td>TAT was also found correlated with disease severity. Meanwhile, there was significant difference of TAT in the death and survival group [52].</td>
</tr>
<tr>
<td>vWF antigen</td>
<td>↑</td>
<td>Severe versus non-severe</td>
<td>vWF: Ag is a relevant predictive factor for in-hospital mortality in COVID-19 patients [53].</td>
</tr>
<tr>
<td>FVIII activity</td>
<td>↑</td>
<td>ICU versus non-ICU</td>
<td>High FVIII activity was observed in patients with COVID-19 who are admitted to the ICU [54].</td>
</tr>
<tr>
<td>PAI-1</td>
<td>↑</td>
<td>COVID-19 versus healthy control</td>
<td>Markedly elevated t-PA and PAI-1 levels were observed in patients hospitalized with COVID-19. High levels of t-PA and PAI-1 were associated with worse respiratory status [55].</td>
</tr>
<tr>
<td>sP-selectin</td>
<td>↑</td>
<td>ICU versus non-ICU</td>
<td>Plasma concentrations of P-selectin are also significantly elevated in patients with COVID-19 who are admitted to the ICU [54].</td>
</tr>
<tr>
<td>sTM</td>
<td>↑</td>
<td>ICU versus non-ICU</td>
<td>In patients, soluble thrombomodulin concentrations greater than 3.26 ng/mL were related to lower rates of hospital discharge [54].</td>
</tr>
<tr>
<td>sCD40L</td>
<td>↑</td>
<td>ICU versus non-ICU</td>
<td>In COVID-19 patients, sCD40L levels were higher in cases requiring admission to intensive care unit [56].</td>
</tr>
</tbody>
</table>

Arrows indicate the direction of change (s = increase, at the direction of change (sCOVID-19) with respect to a control group or reference range defined in the comparator column. ICU: intensive care unit; CRP: C reactive protein; COVID-19: coronavirus disease 2019; TAT: thrombin-antithrombin; vWF: von Willebrand factor; PAI-1: plasminogen activator inhibitor-1; sP-selectin: soluble P-selectin; sTM: soluble thrombomodulin; sCD40L: soluble CD40 ligand.
Pulmonary pathophysiology: Pulmonary post-mortem studies of COVID-19 patients have shown capillary congestion, intra-alveolar edema, necrosis of pneumocytes, and type 2 pneumocyte hyperplasia [22]. Capillary congestion can progress to pulmonary hypertension (PH). Additionally, other underlying mechanisms of PH in patients with COVID-19 exist. First, vascular occlusion resulting from microthrombi gives rise to significant increases in pulmonary vascular resistance, facilitating the formation of PH. Second, SARS-CoV-2 infection downregulates ACE2 expression, which increases angiotensin II (Ang II), further resulting in pulmonary vasoconstriction by binding with Angiotensin II type 1 receptor (AT1) and pulmonary hypertension. Extensive ECs damage has been observed in COVID-19 patients. In addition to SARS-CoV-2 direct damage, the downregulation of ACE2 expression results in activation of the des-Arg9 bradykinin/bradykinin receptor B1 axis, increasing vascular permeability. High cytokines levels, including IL-6, IL-1β, lead to increased ECs contractility and the relaxation of inter-endothelial junctions [23]. NETs decrease expression of the inter-cellular (junctional) proteins CD31 and VE-cadherin, which disturbs the integrity of the ECs monolayer. Profound hypoxia resulted from capillary congestion magnifies ECs damage, blood plasma into pulmonary alveolar, and hypercoagulability by increasing blood viscosity, reactive oxygen species (ROS) and activating HIF-1α [24]. Hypoxia-induced ROS production directly leads to ECs injury. Additionally, the increase of chemokine stromal cell-derived factor-1 (SDF-1, CXCL12) induced by HIF-1α results in monocyte migration and recruitment to lung tissue by CXCR4 receptor [25]. Macrophages in lung tissues are polarized into M1 macrophages and extensively injure ECs by the release of proinflammatory cytokines. Due to widespread ECs injury, intravascular substances, consisting of plasma, fibrinogen, albumin and globulin, extrude into alveolar lumens at high pulmonary artery pressure. Alveolar spaces are often filled with plasma, aggravating the mismatch of ventilation and perfusion, which is a hallmark feature of COVID-19 ARDS. NETs, acting on macrophages, lead to excessive cytokine production and more immune cells recruited into the lungs, resulting in diffuse alveolar damage [26]. Owing to difficulty breathing and low blood oxygen saturation levels, patients with severe COVID-19 require mechanical ventilation to improve alveolar oxygenation. Mechanical ventilation takes away substantial water from the plasma in the alveoli, leading to highly concentrated plasma and gelatinous protein formation. Subsequently, plasma constantly intrudes into the alveolar lumens. The pathogenetic condition of COVID-19 patients sharply deteriorates, associated with high mortality (Figure 1).

Thrombosis: Massive thrombosis is commonly found in this phase. Numbers of SARS-CoV-2 are released into the blood, then enter endothelial cells as the disease progresses. Proinflammatory cytokines released by activated blood cells and NETs together lead to extensive EC damage. Damaged ECs further result in the disruption of vessel wall integrity and slow blood flow by regulating vasoconstriction. Substantial amounts of blood cells and coagulation factors accumulate in the site of injured vessel. Cytokines-induced increased expression of P-selectins and von Willebrand factor on damaged ECs recruits and adheres leukocytes (mainly neutrophils) and platelets (PLTs). Furthermore, in severe inflammatory states, a secondary deficiency of a disintegrin and metallo-
Figure 1. The role of NETs in the pathophysiology of ARDS with COVID-19 patients. (1) Proposed mechanisms of PH. Capillary congestion and significant increases in pulmonary vascular resistance induced by microthrombi can progress to pulmonary hypertension. Additionally, SARS-CoV-2 infection downregulates the expression of ACE2, which increases angiotensin II, resulting in pulmonary vasoconstriction by binding with Angiotensin II type 1 receptor and pulmonary hypertension. (2) Proposed mechanisms of EC damage. In addition to SARS-CoV-2 direct damage, the downregulation of ACE2 expression results in activation of the DABK/BKB1R axis, increasing vascular permeability. High cytokine levels, including IL-6, and IL-1β, result in increased ECs contractility and the relaxation of inter-endothelial junctions. NETs induce decreased expression of inter-cellular (junctional) proteins CD31 and VE-cadherin, which disturb the integrity of the ECs monolayer. (3) Hypoxia mechanisms of ECs damage. Profound hypoxia resulted from capillary congestion magnifies ECs damage and hypercoagulability by increasing blood viscosity, ROS and activating HIF-1α. Hypoxia-induced ROS production directly contributes to EC injury. Additionally, HIF-1-induced increase of SDF-1 (CXCL12) contributes to monocytes migration by binding with CXCR4 receptor. Migrating monocytes can be polarized into M1 macrophages and release substantial amounts of cytokines to injure ECs. (4) Proposed mechanisms of inflammation. NETs, acting on macrophages, lead to excessive cytokine production and more immune cell recruitment into the lungs, resulting in hyperinflammatory states and even diffuse alveolar damage. Abbreviations: PH: pulmonary hypertension; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ACE2: angiotensin-converting enzyme 2; Ang II: Angiotensin II; IL: interleukin; TNF: Tumor necrosis factor; ECs: endothelial cells; DABK/BKB1R axis: des-Arg9 bradykinin/bradykinin receptor B1 axis; HIF-1α: Hypoxia-Inducible Factor-1α; mTOR: mammalian target of rapamycin; NETs: neutrophil extracellular traps; CXCL: CXCL: chemokines IL; SDF-1: stromal cell-derived factor-1; LL-37, the antimicrobial peptide LL-37; MPO, myeloperoxidase; M1, M1 macrophages; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; PLT: Platelet; RBC: Red blood cell PMN: Polymorphonuclear neutrophil.
Protease with a thrombospondin motif repeats 13 (ADAMTS13) leads to reduced vWF cleavage and pathologically increases platelet-vessel wall interaction [27]. Microparticles (MPs) of activated PLTs, expressing high mobility group box 1, result in the formation of NETs by autophagy pathway, which entraps more PLTs and red blood cells (RBCs) [28]. Antimicrobial peptides LL-37 of NETs in turn promote platelet activation in a P-selectin-dependent manner, therefore form a vicious circle and further exacerbate the formation of thrombosis (Figure 2).

DNA and histones, the main components of NETs, also enhance thrombus formation. DNA induces the formation of factor XIIa (FXIIa), then activates pre-kallikrein (PK) and factor XI (FXI), and ultimately initiates the generation of fibrin. Histones upregulate TF expression on ECs and monocytes to a level that triggers the production of plasma thrombin. Histones also induce phosphatidylserine (PS) exposure on PLTs and RBCs, thereby supporting assembly of the prothrombinase complex [29] (Figure 2).

Microparticles (MPs) are membrane-bound vesicles released after activation or apoptosis of blood cells, ECs, and tumor cells. Cytokine storm leads to apoptosis of the blood cells (mainly PLTs, RBCs, and white blood cells), which release amounts of MPs and subsequently form the MP storm [30]. PS exposure on MPs enhances the binding and formation of FVIIa, FXa, FX, and thrombin by interaction with the Gla domains within the proteins and facilitates FVaXa and TF activity [31]. MPs attach to NETs, which provide a scaffold for MP coagulation, and the PS on MP binding with FVaXa and TF, thereby leading to systemic thrombosis [32] (Figure 2).

The levels of TF expression and PS exposure on ECs after treatment with NETs are increased. Moreover, ECs cultured under high concentration of NETs can induce increased FXa complex and thrombin [33]. PS exposure on ECs provides platforms for the binding of clotting factors, further promoting thrombus by the intrinsic pathway. Tissue factor (TF) on EC binding to
factor VIIa (FVIIa) initiates coagulation by the extrinsic pathway. The role of PS may have an advantage over TF in COVID-19 associated thrombosis. First, PS exposure is observed on all activated or apoptotic cells, while TF is mainly expressed on monocytes and ECs in the pathologic thrombotic process [34]. Secondly, severe damage of monocytes and ECs, and even apoptosis in a hyperinflammatory state result in decreased synthesis and expression of TF, and PS exposure on blood cells is unaffected and conversely increased. Thirdly, most TF is in the inactive state and requires PS-dependent decyption [35]. Additionally, EC inflammation triggered by NETs accompanies high levels of FVIII and vWF:Ag, which is related to thrombosis [24] (Figure 2).

Hypoxia increases the transcription of HIF1α and HIF2α subunits. The underlying mechanisms of HIFs in thrombus formation are multiple. First, HIF1α upregulates neutrophil stress-response protein REDD1, leading to NET release by autophagy-associated signaling pathways. NETs provide a scaffold for immune cells, coagulation factors, and MPs, accordingly enhancing thrombosis. Secondly, platelet HIF2α mediates the expression of plasminogen activator inhibitor-1 (PAI-1) and MP release [36]. The levels of PAI-1 are elevated, which marks decreased fibrinolytic activity in COVID-19 patients, leading to the imbalance of coagulation and fibrinolysis. Thirdly, on ECs, hypoxia-mediated HIF2α reduces expression of tissue factor pathway inhibitor (TFPI), which suppresses extrinsic pathways of coagulation, resulting in pro-thrombotic phenotype [37] (Figure 2).

In addition to the coagulation system, an exhausted fibrinolytic system may affect thrombus formation. Fibrinolytic system is initiated by frequent thrombus formation and markedly increased plasmin, which dissolves fibrin-rich blood clots, then generates high levels of D-dimer, indicating a high fibrinolytic state. Subsequently, excessive consumption of plasmin leads to decreased fibrinolytic function. This explains why hyperfibrinolysis characterized by elevated D-dimer and fibrinolysis inhibition with elevated PAI-1 simultaneously exist.

Acute respiratory distress syndrome: The presentation of severe COVID-19 is progressive hypoxemia and dyspnea, and often patients require mechanical ventilation support in this phase. Pulmonary computed tomography (CT) shows that the surrounding ground-glass opacity in lung tissue conforms to the Berlin criteria of ARDS in COVID-19 patients [38]. The pathophysiology of ARDS induced by SARS-CoV-2 is similar to that of severe community-acquired pneumonia induced by other bacteria or viruses [25]. Various animal models of the agents responsible for ARDS agree that neutrophils have a central role in early innate immune response, and excessive neutrophils accumulate in the pulmonary capillaries and alveoli of ARDS patients, which has been shown in recent autopsy reports of COVID-19 patients. Neutrophils have been closely correlated with the development of ARDS, and NETs are regarded as primary effector cells of tissue injury [4]. Cytokine storm induced by NETs and macrophages, leads to excessive cytokines and more immune cell recruitment into the lungs, resulting in diffuse alveolar damage and even ARDS [26].

Multiple organ failure: Systemic hyperinflammation induces the increase of NETs. Circulating histones have been shown to cause alveolar capillary occlusion, toxicity in lung tissue, and activated coagulation cascade, which ultimately leads to lung injury and MOF [39]. As symptoms of SARS-CoV-2-induced sepsis patients progress, high levels of cf-DNA result in MOF [40]. Massive cytokines induce extensive EC damage and dysfunction, further aggravating thrombus formation. Extensive thrombosis spreads throughout the body and induces an inadequate blood supply to vital organs, resulting in both hypoxemia and hypoglycemia and subsequent damage and failure of multiple organs.

A marked decrease of lymphocytes in COVID-19 patients is observed in this phase, suggesting a decline in adaptive immunity. Lymphopenia is a significant factor correlated with the severity and mortality of the patients [41]. In addition to lymphocyte death caused by the interaction of activated Fas and Fas ligand and the TNF-related apoptosis-induced ligand axis, which initiates or promotes the death of lymphocytes. Lymphocyte infection with SARS-CoV-2 results in decreased lymphocytes [42]. Lymphocyte entrapping by NETs can also account for lymphopenia [43]. The ECs adhere to blood cells and clotting factors, which cause uncontrolled clotting. In response to extensive thrombosis,
the body undertakes measures to dissolve fibrin-rich blood clots, explaining why increased fibrin degradation products (D-dimers) predict poor prognosis in COVID-19 patients [44]. Additionally, EC damage induced by inflammation results in pathologic release of urokinase-type plasminogen activator (u-PA), which is also the reason for increased D-dimer. The levels of soluble thrombomodulin, soluble P-selectin, as well as soluble CD40 ligand are elevated, which mark EC injury and PLTs activation [24] (Table 1).

**Theoretical treatment strategy**

Owing to microthrombi in the initial infection phase, early effective antithrombotic therapy combined with full-dose alteplase for systemic fibrinolysis can maintain blood flow patency, which is the key to inhibit severe diseases caused by SARS-CoV-2 and improve prognosis. Antithrombotic therapy consists of antiplatelet therapy (aspirin, clopidogrel) and anticoagulation, including unfractionated heparin, low-molecular-weight heparins (LMWH), direct oral anticoagulants (DOACs), Xla, and Xlla inhibitors. Heparin not only exhibits anticoagulant actions by the inhibition of thrombin production, but also exerts an anti-inflammatory effect by the downregulation of IL-6, and thus unfractionated heparin or LMWH remains the best choice of anticoagulant for admitted patients [45, 46]. DOACs, including Ila and Xa inhibitor, in addition to the antithrombotic effect, also attenuate the inflammatory response, which shows beneficial effects for COVID-19 patients. FXII and FXI are located at the initiation of the clotting cascade and critical for the propagation and stabilization of thrombus but less important for hemostasis. Therefore, FXIIa and FXlla inhibitors decrease the consumption of coagulation factors and the levels of D-dimer, as well as not increase bleeding risk. Treatment with antiviral drugs can inhibit viral production in this stage, including remdesivir, lopinavir/ritonavir (LPV/RTV). NE is involved in S protein cleavage and virus invasion, and treatment with sivelestat is beneficial for COVID-19 patients (Figure 3).

In the second phase, the continuation of the antithrombotic therapy with fibrinolytic therapy is necessary for COVID-19 hospitalized patients to improve prognosis. Treatment with mechanical ventilation to improve hypoxia is a vital step in this stage, which suppresses hypoxia-related thrombosis, inflammation, and tissue damage, delaying disease progression. The targeting therapy of NETs is considered to be an appropriate therapeutic method to suppress NET-mediated inflammation, cytokines release, and lung injury during the second phase. Early use of cytokine antagonists, including anakinra (an IL-1 receptor antagonist) and tocilizumab (a humanized monoclonal anti-IL-6 receptor antibody), can reduce infiltration of inflammatory cells in the lungs, which is the key to cytokine storm in COVID-19 patients [47]. However, therapeutic strategies targeting the hyperactive cytokines with cytokine antagonists must be balanced with sustaining enough inflammatory response for pathogen clearance. Additional drugs that attenuate inflammation can also be used at this stage (Figure 3).

Given that thrombotic complications are central determinants of the high mortality rate in COVID-19, antithrombotic therapy with thrombolytic therapy is of critical importance in the hyperinflammation phase [48]. Owing to fibrin deposition in the alveoli seriously disturbing gas exchange, COVID-19 patients use alteplase by inhalation to improve respiratory symptoms and hemodynamics. COVID-19 patients still require mechanical ventilation in this stage. The emerging view of the important role of ECs indicates that the therapy to improve ECs function, including statins, can slow down its progression and reduce mortality in COVID-19. Cytokine antagonists, including IL-1β, IL-6, are regarded as an attractive therapeutic strategy for targeting cytokine storm. It is necessary to target NETs to improve outcomes of COVID-19 patients (Figure 3).

Severe and critical COVID-19 patients are more likely to develop thrombosis after discharge. We recommend to receive long-term oral anticoagulants apixaban. Moreover, patients are required to regularly monitor thrombosis and spontaneous bleeding tendency.

**Conclusion**

NETs are involved in pulmonary pathophysiology, inflammation, and thrombus formation of COVID-19. SARS-CoV-2 infection causes the damage of alveolar epithelial cells, which leads to neutrophil infiltration and NETs formation in the lungs. Pulmonary hypertension, extensive
EC damage, fibrin deposition, and activated immune cells are involved in pulmonary pathophysiologic processes and result in sharp deterioration. NETs provide a scaffold for coagulation factors, blood cells, and high amounts of MPs described as microparticles storm, promoting thrombosis. Based on disease characteristics and mechanisms of different stages, we provide corresponding antithrombotic therapy, systemic fibrinolysis, cytokine antagonists,
and targeting NET therapy. We highlight the importance of early antithrombotic therapy with systemic fibrinolysis, which dissolves intravascular microthrombi, alleviating and arresting the progression of the disease in the early stage. Meanwhile, NETs not only predict the severity of COVID-19, but also serve as a therapeutic target for COVID-19.

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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; IL-6, Interleukin-6; TNF, tumor necrosis factor; IL-1β, Interleukin-1β; ARDS, acute respiratory distress syndrome; MOF, multiple organ failure; ECs, endothelial cells; NETs, neutrophil extracellular traps; S protein, Spike protein; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2; NE, neutrophil elastase; HIF-1α, hypoxia inducible factor-1α; ROS, reactive oxygen species; SDF-1, stromal cell-derived factor-1; CXCL, chemokines IL; TLR2, toll-like receptor 2; TF, tissue factor; CRP, C-reactive protein; PT, prothrombin time; FDPs, fibrinogen degradation products; vWF, von Willebrand factor; AT, antithrombin; FVIII, factor VIII; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3; PH, pulmonary hypertension; Ang II, angiotensin II; AT1, Angiotensin II type 1 receptor; PLTs, platelets; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin motif repeats 13; MPs, microparticles; RBCs, red blood cells; FXIIa, active factor XIIa; PK, pre-kallikrein; FXI, factor XI; PS, phosphatidylserine; FVIIa, active factor VIIa; MPVs, microparticles; PAI-1, plasminogen activator inhibitor-1; TFPI, tissue factor pathway inhibitor; u-PA, urokinase-type plasminogen activator; DOACs, direct oral anticoagulants; LPV/RTV, lopinavir/ritonavir.

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