Am J Transl Res 2021;13(5):3925-3941
www.ajtr.org /ISSN:1943-8141/AJTR0127408

Review Article

Mechanisms of COVID-19 thrombosis in an inflammatory environment and new anticoagulant targets

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Received December 1, 2020; Accepted February 25, 2021; Epub May 15, 2021; Published May 30, 2021

Abstract: COVID-19 is widely epidemic in the world and poses a great threat to our life. Coagulopathy is one of the major characteristics in the COVID-19 patients. A growing number of studies have found that the severe COVID-19 patients have thrombotic microangiopathy and thromboembolism. Coagulopathy associated with increased risk of death in the patients. Unfortunately, the mechanism of coagulopathy is not clearly addressed. Understanding the pathophysiological mechanism of COVID-19 thrombosis and improving the coagulopathy through efficient treatment may help to stop disease progression, reduce mortality and sequelae. In severe COVID-19 patients, inflammation, cytokine storm, and coagulation are closely related, which together cause blood congestion and thrombosis. Many cytokines activate blood cells, expressing activating factors or releasing activated microparticles, and then accelerating thrombosis. However, the role of blood cells is not well understood in COVID-19 patients. In addition, cytokines stimulate endothelial cells, transforming them into a procoagulant phenotype. Therefore, determine their role and propose new strategies for the prevention and treatment of thrombosis in severe COVID-19 patients. We outline the major events of coagulopathies, discuss the role of blood and endothelial cells in thrombosis, to formulate a new anticoagulation protocol.

Keywords: COVID-19, thrombosis, cytokines, phosphatidylserine, neutrophil extracellular traps, anticoagulant therapy

Introduction

Since the outbreak of coronavirus disease-2019 (COVID-19) in December 2019, it has spread widely around the world. The disease, caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), shows flu-like symptoms and viral pneumonia, and develops into acute respiratory distress syndrome (ARDS), even multiple organ failure [1, 2]. The virus invasion stimulates the activation of the immune system, causing release of excessive pro-inflammatory factors, forming a cytokine storm in severe and critical patients, which is closely related to high mortality of COVID-19 patients [3-5]. There is a wide range of interactions between inflammation and coagulation. The activation of one system may amplify the effect of another system, forming inflammatory-thrombosis exacerbating disease progression [1, 6, 7].

Studies have shown that thrombosis is one of the main causes of death in COVID-19 patients. In critically ill patients, D-dimer, fibrin degradation products, and fibrinogen are significantly increased, and D-dimer can be used as an indicator to predict patient mortality [8-10]. Early application of low-molecular-weight heparin (LMWH) improved the prognosis of patients, reduced mortality. Active anticoagulation measures were benefit to the clinical treatment of COVID-19 patients. Unfortunately, thrombotic events still occur in some patients with using

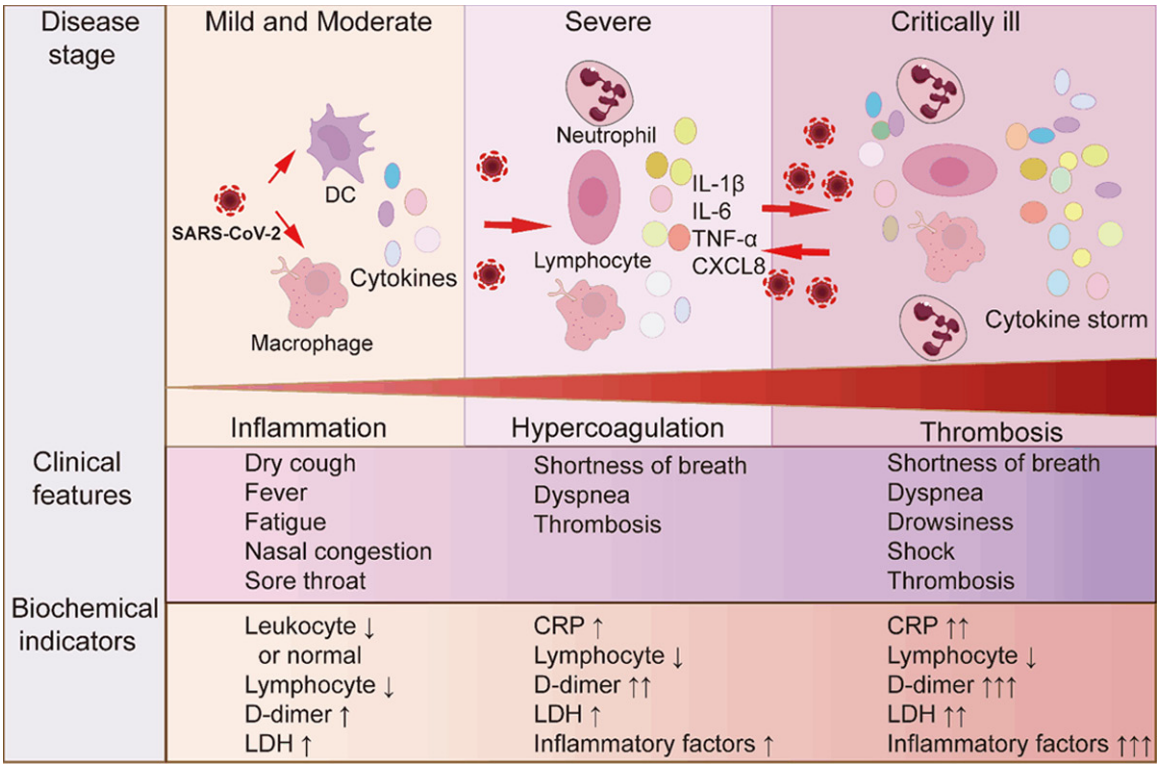

Figure 1. Clinical features and biochemical changes following COVID-19 infection. After virus invasion, active defense is made in response to the virus. With the progression of disease severity, patients show characteristic changes in clinical manifestations and hematological biochemical indicators. CRP: C-reactive protein; DC: Dendritic Cells; IL: interleukin; LDH: Lactate dehydrogenase; PMN: Polymorphonuclear neutrophil; TNF: tumor necrosis factor.

prophylactic anticoagulant [11-13]. This indicates that the thrombosis mechanism is complex, and other mechanisms are involved, so it is necessary to further explore the mechanism of coagulation disorders in COVID-19 patients. A variety of cells are involved in the process of thrombosis, and cytokines promote the activation of blood cells, thus we mainly explore the role of blood cells in thrombosis of COVID-19 patients. To expect multi-target blocking of thrombosis, improve disease prognosis, reduce patient sequelae and mortality.

Clinical feature of COVID-19

Coronavirus, a respiratory virus, is a coated RNA virus which induces the common cold to Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). Eventually, it will develop to fatal lower respiratory infections and extrapulmonary manifestations [4]. SARS-CoV-2, known as COVID-19, is a newly discovered coronavirus in 2019. It is a spherical particle with a diameter of about 50 nm, which consists of single-stranded RNA, the surface cell membrane, envelope protein, and nucleocapsid. The "spike" glycoprotein, also named S protein, has a high affinity for a receptor, angiotensin-converting enzyme 2 (ACE2). ACE2 is high expression mainly in alveolar cells, myocardial cells, endothelial cells (ECs) and others. COVID-19 can entry the lung, heart, blood vessels, kidney and gastrointestinal tract cells through the ACE2, leading to tissue injury, organ failure, such as ARDS, myocardial damage, kidney damage [14, 15].

COVID-19 is mainly spread by droplets, enters the lungs through the respiratory tract. Macrophages engulf the virus to secrete cytokines and promote the recruitment of white blood cells in the lung. In the early stage, the patient was in an asymptomatic stage or has a mild dry cough, fever, fatigue, and D-dimer increased slightly (**Figure 1**) [16]. With the virus proliferating, the immune system reacts strongly, appearing a series of clinical symptoms. The patient developed symptoms such

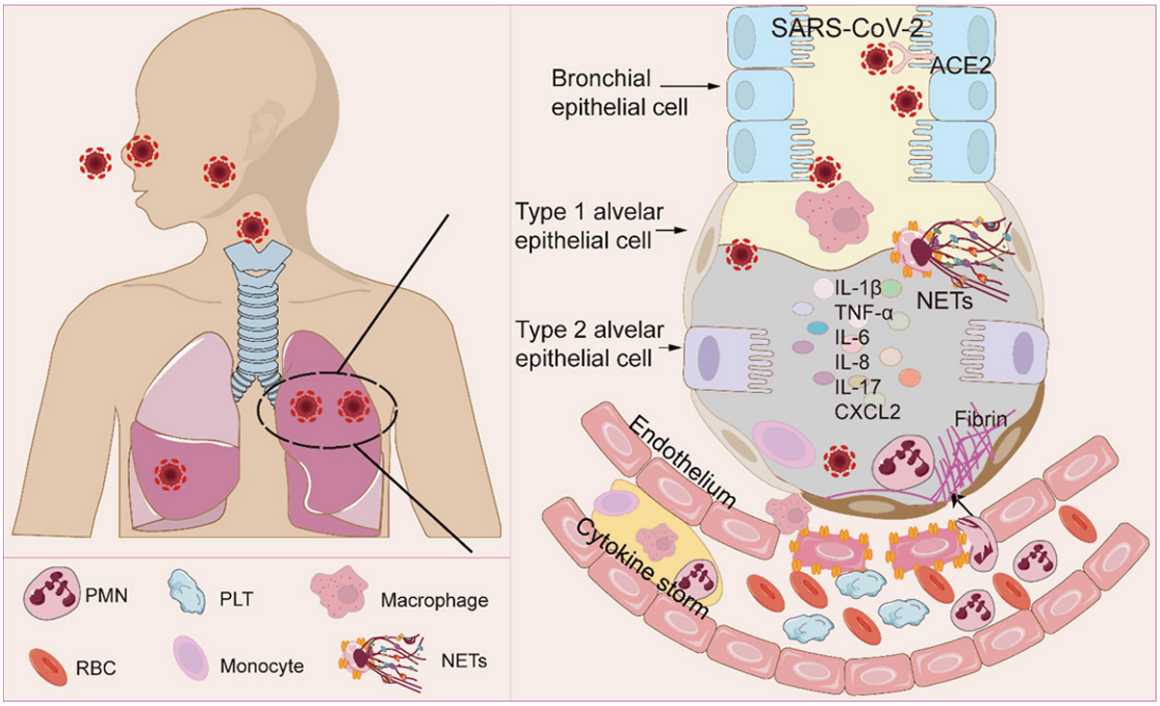

Figure 2. The mechanisms of COVID-19 damages to the lung. The COVID-19 invades the lung through the respiratory tract, enters the alveolar epithelial cells via the ACE2 receptor, activates the immune system, secretes pro-inflammatory factors and chemokines, which recruit leukocyte and promote virus clearance. However, as the number of virus increases, immune cells continue to secrete cytokines and promote the formation of cytokine storm. In patients with severe COVID-19, the virus invades blood vessels, damages vascular endothelial cells, increases vascular permeability, causes cells and proteins to enter the alveoli, accelerates alveolar endothelial cell damage and fiber deposition, and aggravates alveolar damage, forming a vicious circle. ACE2: angiotensin-converting enzyme 2; CXCL: Chemokines; IL: interleukin; NETs: neutrophil extracellular traps; PLT: Platelet; PMN: Polymorphonuclear neutrophil; RBC: Red blood cell; TNF: tumor necrosis factor.

as shortness of breath, dyspnea, shock and thrombosis. The levels of CRP, LDH, cytokines, D-dimer are increased significantly, which lead to coagulopathy [2, 17, 18].

The autopsy revealed that there was a large amount of inflammatory cells infiltration in the patient's lung, alveolar damage, and capillary congestion. Extensive fibers deposits and microthrombus formation were seen at the injury site, these conditions are more common in critically ill patients. In addition, quantities of infiltrating inflammatory cells secreted cytokines, which promoted the recruitment of inflammatory cells, then cytokines caused tissue and endothelial cells damage. The broken connection between damaged endothelial cells, coupled with vascular congestion, caused cells and proteins to infiltrate into the alveolar cavity, aggravating alveolar damage and fiber deposition, resulting in a worsening of the disease (**Figure 2**).

Cytokine storm

When SARS-CoV-2 invades, the immune system responds quickly, releasing pro-inflammatory cytokines and chemokines to promote defense against the virus [19]. Tamim et al. divided this process into stage I (early stage), stage II (IIa pulmonary symptom without hypoxia, IIb pulmonary symptom with hypoxia), stage III (hyper-inflammation period) [20]. He believed virus amplified and multiplied in the body, stimulating immune cells to release many pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α), and formed cytokine storm in severe and critically ill patients.

IL-6 was positively correlated with COVID-19 progression and significantly increased in critically ill patients, up to about 10 times compared with normal people or mild patients, which was closely related to the prognosis of

Table 1. Association between coagulation abnormalities or markers of thrombosis

Study	Group	Leucocytes ($\times10^9$/L)	Neutrophil ($\times10^9$/L)	D-dimer (μg/mL)	Fibrinogen (g/L)	CRP (mg/L)	APTT (s)
Zhang et al. [9] (n=343)	D-dimer <2.0	6.5±4.4	3.5 (2.7, 4.8)	0.4 (0.2, 0.7)	4.1 (3.1, 5.1)	1.7 (0.3, 16.6)	29.6±4.3
	D-dimer >2.0	7.4±3.7	4.7 (3.4, 7.3)	4.8 (3.0, 11.9)	4.3 (3.2, 5.6)	13.6 (1.8, 62.8)	28.8±5.2
	P value	0.09	<0.001	<0.001	0.54	<0.001	0.21
Cui et al. [33] (n=81)	VTE (n=20)	7.8±3.1	NR	5.2±3.0	NR	NR	39.9±6.4
	Non-VTE (n=61)	6.6±2.6	NR	0.8±1.2	NR	NR	35.6±4.5
	P value	0.12	NR	<0.001	NR	NR	0.001
Wang et al. [34] (n=65)	Mild (n=30)	5.2 (2.4)	3.8 (2.4)	1.6 (3.0)	NR	53.6 (57.7)	NR
	Severe (n=20)	6.9 (3.8)	5.7 (3.7)	4.7 (7.4)	NR	91.8 (77.8)	NR
	Extremely severe (n=15)	8.7 (4.1)	7.7 (3.9)	6.9 (8.4)	NR	114.9 (62.5)	NR
	P value	<0.001	<0.001	<0.001	NR	<0.01	NR
Han et al. [35] (n=134)	Controls (n=40)	NR	NR	0.3±0.2	2.9±0.5	NR	28.7±3.0
	SARS-CoV-2 (n=94)	NR	NR	10.4±25.3	5.0±1.5	NR	29.0±2.9
	P value	NR	NR	<0.001	<0.001	NR	<0.001
Yang et al. [36] (n=93)	non-severe (n=69)	6.4±2.4	4.55±0.21	0.5±0.4	NR	20.1±24.5	NR
	severe (n=24)	9.1±5.6	7.73±5.4	16.6±23.1	NR	53.9±60.1	NR
	P value	<0.01	<0.01	<0.01	NR	<0.01	NR

APTT, activated partial thromboplastin time; CRP, C-reactive protein; NR, no report; VTE, Venous Thrombus Embolism.

the disease [5]. IL-6 can promote the macrophage activation, then leading to release multiproinflammatory cytokines and recruit leucocyte and fibroblasts into the lung. This makes the deposition of fibrin, aggravating damage of the lung [21].

IL-17 is a member of the multifunctional cytokine family, mainly produced by activated T cells, neutrophils and mast cells, and participates in a variety of inflammatory diseases and autoimmune diseases [22-24]. Under physiological conditions, IL-17 is responsible for skin and mucosal immunity of the mouth, lung, airways, gastrointestinal tract, and vagina, where these organs widely express IL-17 receptors. It induces mucosal epithelia secrete antimicrobial peptides to maintain the epithelial barrier, directly removing pathogens or preventing microbial invasion [25, 26]. Regrettably, excessive IL-17 aggravates the inflammatory level and induces the airway epithelium to secrete chemokine ligand (CXCL) 1, CXCL5, IL-8, IL-6, granulocyte colony stimulating factor (GCSF) and granulocyte and macrophage colony stimulating factor (GM-CSF), recruiting granulocytes to infiltrate into the lung [27, 28]. In the animal model of lipopolysaccharide (LPS) induced ARDS in mice, IL-17 was increased the severity of lung injury and closely related to the low survival rate of mice. Excessive IL-17 accelerated the severity of lung injury, which is closely related to the low survival rate of mice [29]. The level of IL-17 was positively associated with mortality in severely ill COVID-19 patients [30]. IL-17 promotes the release of various proinflammatory factors, and inflammatory cytokines can magically promote the production of IL-17 [31]. The interaction of IL-17 and proinflammatory cytokines form a vicious circle, which may lead to accelerate the formation of COVID-19 cytokine storm. Due to the close relationship between inflammation and coagulation, we next explore the "contribution" of the cytokine storm formed by COVID-19 in thrombosis.

Abnormal coagulopathy in COVID-19

Most studies have found that D-Dimer, C-reactive protein (CRP) and fibrinogen are significantly increased in COVID-19 patients. D-dimer was positively related to the severity of the disease and can predict the risk of deep vein thrombosis and patient death [8, 9, 32]. **Table 1** summarizes the current specific changes in different coagulation parameters after infected [9, 33-36]. As the severity of the disease progress, the coagulation indicators were increased significantly in COVID-19 patients. This makes the clinical administration of COVID-19 patients a reasonable basis for using anticoagulation therapy, and by monitoring changes in laboratory indicators, timely adjustment of anticoagulant dose.

In severe COVID-19 patients, through comparing patients with or without LMWH for antico-

agulation treatment, and observing the 28-day mortality, they found that when critical patients meet the SIC score >4 or D-dimer >6 times the normal level, anticoagulant therapy could improve the prognosis and reduce their mortality in critically ill patients [11]. Unfortunately, some patients still have a high incidence of thrombosis during using of anticoagulants such as LMWH [37]. **Table 2** lists some studies of patients who experienced thrombosis with or without anticoagulation. Although most patients were treated with prophylactic anticoagulation, thromboembolism of varying degrees still occurred, and bleeding happened in some patients [12, 33, 37-41]. Consequently, the exploration of the thrombosis mechanism of COVID-19 may improve the effectiveness of anticoagulation, reduce the risk of bleeding.

The mechanism of thrombosis

Microparticle storm

Phosphatidylserine (PS), a negatively charged phospholipid, is one of the major phospholipid components, which makes up the cell membrane and usually locates in the intracellular membrane. When the cell is stimulated or activated or apoptosis, the cell membrane occurs to remodel, then PS is flipped from intracellular to extracellular [42]. It is the main cofactor for hemostasis and thrombosis. There are two PS-recognition motifs found among coagulation factors: the Gla domain and discoidin-like C2 domain [43, 44]. Factors VII, IX, X, and prothrombin, contain an N-terminal Gla domain and factors V and VIII have a C2 domain responsible for targeting to PS. The two domains will provide a binding site for activated factor X (FXa) and prothrombin complexes to promote thrombosis [43, 45]. Microparticles (MPs) are that cells remodel during apoptosis or activation, releasing a small vesicle, about 100-1000 nm, and contain large amounts of PS [46, 47]. Studies showed that MPs have a variety of biological activities and play a role in coagulation, inflammation, angiogenesis and intercellular communication [48-50]. In our previous study, we found that leukocytes, red blood cells, and monocytes release large amounts of MPs during the active phase in patients with inflammatory bowel disease, through labeling cell-specific expression molecules and using flow cytometry to determine the source of MPs in the blood [51]. In patients with oral squamous cell carcinoma, especially patients with stage III/IV, many procoagulant MPs were detected in the circulation [52]. MPs plays an important role in the formation of procoagulant state in sepsis patients [53].

IL-6 and IL-8 is associated with changes in the membrane structure of red blood cells and platelets. Pre-blocking IL-6 and IL-8 would reduce the level of MPs [54]. Unfortunately, there are many cytokines in COVID-19 patients, and cytokine storm are formed in severe and critically ill patients. Abnormal hypercoagulability and thrombosis can be seen in ICU patients and non-surviving patients, which make us to guess the formation of thrombus may be due to a large number of cytokines stimulating circulating blood cells, causing the activation of blood cells and even apoptosis, which lead to the release of cell particles, forming a “Microparticle storm” to promote the formation of inflammatory thrombus (**Figure 3**).

It was found that the membrane of MPs contains the tissue factor (TF). TF combines with activated factor VII (FVIIa) to effectively activate FX and FIX and promote the production of thrombin [55, 56]. Under normal circumstance, TF exists outside the blood vessel, and the presence of TF hardly is detected in circulation to avoid the occurrence of unnecessary coagulation reaction [57]. When the body is stimulated, large amounts of TF will be detected in the blood in a variety of diseases, such as sepsis and angina. Bogdanov VY and his colleagues believed that there were many TF in the blood circulation, and MPs was the main carrier of TF [58]. Normally TF, however, existed in encrypted form, and PS could decrypt the encrypted TF into active TF to participate in thrombosis [59]. In our experiment, the pro-coagulant state was improved after adding anti-TF antibody, and the pro-coagulant activity was significantly reduced when PS was blocked [60, 61]. Thus, we have reasons to believe that PS and TF play a major role in the procoagulant activity of MPs, and PS synergistically promotes the activation of the exogenous coagulation pathway of thrombosis by decrypting TF. Therefore, anticoagulant therapy for COVID-19 can inhibit the formation of thrombin complex by targeted blocking of PS on MPs and blood cells, reducing the occurrence of thrombosis.

Table 2. Studies and main findings for thrombosis events in COVID-19 patients

Study	Patients	Thrombosis type	Main findings	Bleeding events	Therapy
Hanny et al. [37]	400	Venous thrombosis Arterial thrombosis	In critically ill COVID-19 patients, imaging confirmed VTE 10.4% (95% CI, 5.9-16.6%); The incidence in non-critically ill patients was 3.5% (95% CI, 1.6-6.6%). And arterial thrombosis: critical ill COVID-19 3.46%; Non-critical illness 1.3%	The incidence of massive hemorrhage in critically ill patients was 5.6% (2.4-10.7%)	Standard prophylactic dose of ordinary heparin or low molecular weight heparin
Cui et al. [33]	81	Venous thrombosis	Venous thromboembolism occurred in 25% of the 81 critically ill patients	NR	None
Klok et al. [38]	184	Venous thrombosis Arterial thrombosis	VTE accounts for 27% (95% CI 17-37%) of COVID-19 patients in ICU	NR	Use at least a prophylactic dose of anticoagulant
Corrado et al. [39]	362	Venous thrombosis	Incidence of thrombosis was 7.7% in all patients	NR	Prophylactic dose of LMWH
Jean-F et al. [12]	26	Venous thrombosis	Overall VTE rate was 69%. Among COVID-19 patients receiving therapeutic anticoagulation, the incidence of thromboembolic events was high, with 56% VTE, including 6 cases of pulmonary embolism	NR	8 patients received preventive anticoagulation, 18 were treated with therapeutic anticoagulation
Tao et al. [40]	1099	Venous thrombosis	There were 407 patients with COVID-19 who were at high risk for Venous thromboembolism	44 (11%) of 407 also had a high risk of bleeding	Only ten (7%) of 140 patients for whom anticoagulation data were available in our cohort (nine were given heparin and one rivaroxaban)
Bin et al. [41]	48	Venous thrombosis	Lower extremity DVT were detected in 41 patients (85.4%), with 36 (75%) isolated distal DVT and 5 (10.4%) proximal DVT	NR	30-40 mg LMWH

DVT, Deep Venous Thrombus; ICU, Intensive Care Unit; LMWH, Low molecular weight heparin; NR, no report; VTE, Venous Thrombus Embolism.

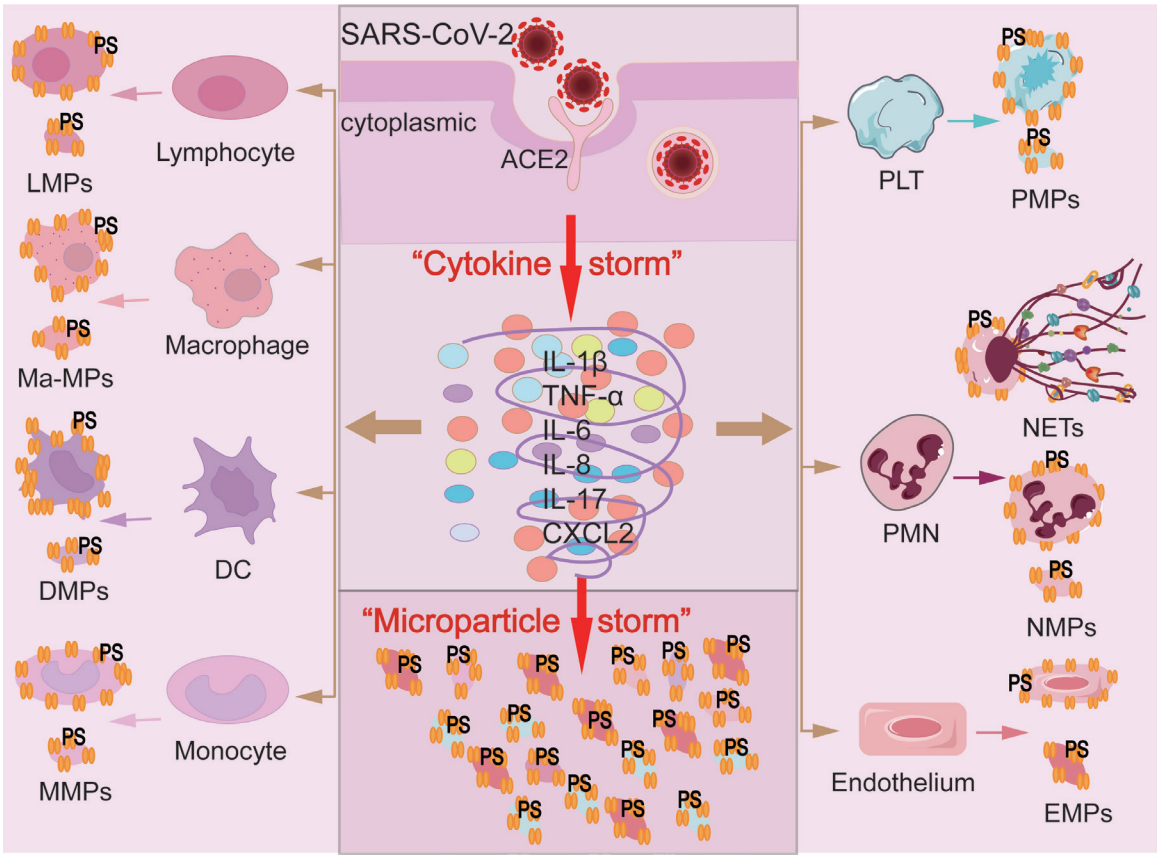

Figure 3. Cytokines trigger microparticle storm. After virus invasion, it stimulates the immune system to release a large number of cytokines and cytokine storm formation, cell factor effects on blood cells (lymphocytes, monocytes, macrophages, dendritic cells, neutrophils and platelets), endothelial cells, the cell activation, large numbers of cells release coagulant activated particles, forming similar cytokine storm "Microparticle storm". ACE2: angiotensin-converting enzyme 2; DC: Dendritic Cells; MPs: Microparticles; PLT: Platelet; PMN: Polymorphonuclear neutrophil; PS: Phosphatidylserine.

NETs promote thrombosis

As a main member of the innate immune system, neutrophils are quickly recruited to the injury site when the body is attacked, playing a defensive role [62]. In 2004, it was first discovered that neutrophils were stimulated and then released a network structure outside the cell, called neutrophil extracellular traps (NETs) [63]. Bacteria, fungi and other microorganisms can stimulate neutrophils to release NETs. NETs consist of a DNA skeleton and a variety of granular enzymes, including histone, myeloperoxidase (MPO), elastase and matrix metalloproteinases [64]. It is a double-edged sword, not only exerts the defensive function of capturing and killing bacteria, but also activates the coagulation cascade reaction, such as the DNA skeleton activates the coagulation contact system to promote coagulation, and the histone inhibits the activation of protein C to promote coagulation [65, 66]. In addition, NETs also cause damage to surrounding tissue, delay wound healing, and aggravate disease progression [67, 68]. Inflammatory factors stimulate neutrophils to release NETs, which are abundant in infection and inflammation sites [63, 68]. IL-8 induces the release of elastase in a concentration-dependent manner, and IL-1β not only facilitate the process of IL-8 stimulating neutrophils, but also induce the release of MPO from neutrophils [69, 70]. TNF-α promotes the respiratory burst of neutrophils, and inflammatory factors stimulate neutrophils to release NETs, which are abundant in infection and inflammation sites [71]. The level of NETs was significantly reduced by neutralizing the effects of inflammatory cytokines through adding antibodies to the plasma of patients [72].

NETs are positively correlated with the severity and survival rate in COVID-19 patients. One

study found that plasma markers of NETs such as cell free-DNA (cf-DNA) and MPO-DNA were significantly elevated in severe and critically ill COVID-19 patients [73]. This study suggests that COVID-19 patients are more likely to produce NETs, and that overall levels of NETs increase with disease progression. The study of Elizabeth et al. reached the same conclusion and made further research. They found a large amount of neutrophil infiltration, NETs and platelet co-aggregation can be seen in the lung through autopsy. The interaction of NETs and platelets promotes the formation of immune thrombosis. Moreover, excessive NETs may further aggravate the lung damage [74]. In addition, the role of NETs in activating the coagulation pathway to promote thrombosis has been widely accepted, adding NETs inhibitors improved blood hypercoagulability and reduced thrombosis in animal models [75, 76]. Therefore, NETs may be involved in the hypercoagulable state and thrombosis in COVID-19 patients. Inhibiting the NETs production, or hydrolyzing its structure, or blocking the granular proteases attached to NETs, may be very helpful in reducing thrombosis in COVID-19 patients.

Endothelial cell damage

Endothelial dysfunction is the main factor of microvascular disease, which changes the balance of the blood vessels to promote vasoconstriction, subsequently leads to organ ischemia, inflammation, and tissue edema, forming a procoagulant state [77]. COVID-19 infects endothelial cells through ACE2 receptor to cause endothelial dysfunction. Autopsy of patients who died of COVID-19 revealed that the lung microvascular endothelial cells were damaged, and the intercellular connection was broken [78, 79]. Numerous of lymphocytes and macrophages infiltration and microthrombus were seen in the lung. In addition, abnormal neovascularization occurred in the lung, and surprisingly the structure of the neovascularization was disordered. Although endothelial cells proliferated in large quantities, they could not arrange in a normal manner, making many neovascularization disturbed and being unable to function normally. Endothelial integrity is an important factor in the prevention of thrombosis. When endothelial cells are injured, they express E-selectin and von Willebrand factor (vWF), which promotes platelet adhesion and aggregation, then form thrombosis [80]. As COVID-19 causes endothelial cell damage and apoptosis, it may induce the release of MPs from endothelial cells, and the exposure of PS on the endothelial cell surface, and promote the binding of coagulation factors, leading to thrombosis. In addition, IL-6 and TNF-α motivate endothelial cells, which facilitate endothelial cells to release procoagulant soluble TF [81]. After COVID-19 infection, the release of pro-inflammatory factors causes endothelial cell damage, which may induce endothelial cells to release MPs, expose PS on the surface of endothelial cell, promote the binding of coagulation factors, and lead to thrombosis, further experiments are needed to confirm our speculation [82, 83].

Previous studies shown that NETs cause endothelial cell damage, especially attached histones, which have a toxic effect on endothelial cells, causing endothelial cells to shrink and activate. The deposition of coagulation factors Xa and Va and fibrin on endothelial cells was observed. By targeting inhibition of histone or hydrolyzation of NETs structure reversed endothelial cell damage on the influence of coagulant activity [72, 84]. Yu Zou and his colleagues compared the two groups of COVID-19 patients who were with mechanical ventilation and no mechanical ventilation, they found that the level of NETs in the mechanical ventilation group was significantly increased, indicating that NETs increased with the severity of the disease [75].

Hypoxia plays a vital role in promoting inflammation and endothelial damage [85]. It promotes the hypoxia-inducible factor (HIF) transcription in the nucleus [86]. HIF promotes the release of inflammatory factors by activating NF-κB pathway and aggravates local inflammation [87]. Hypoxia damages endothelial cell, destroys intercellular connection and the endothelial barrier, then promotes microleakage, high viscous blood, and thrombosis [88]. Moreover, it not only activates the exogenous coagulation pathway, promotes the production of PAI-1 and inhibits the fibrinolytic system, but also inhibits the anticoagulation system [89, 90]. Regrettably, studies have shown that hypoxia is one of the important symptoms of COVID-19 [91, 92]. Although oxygen is used to

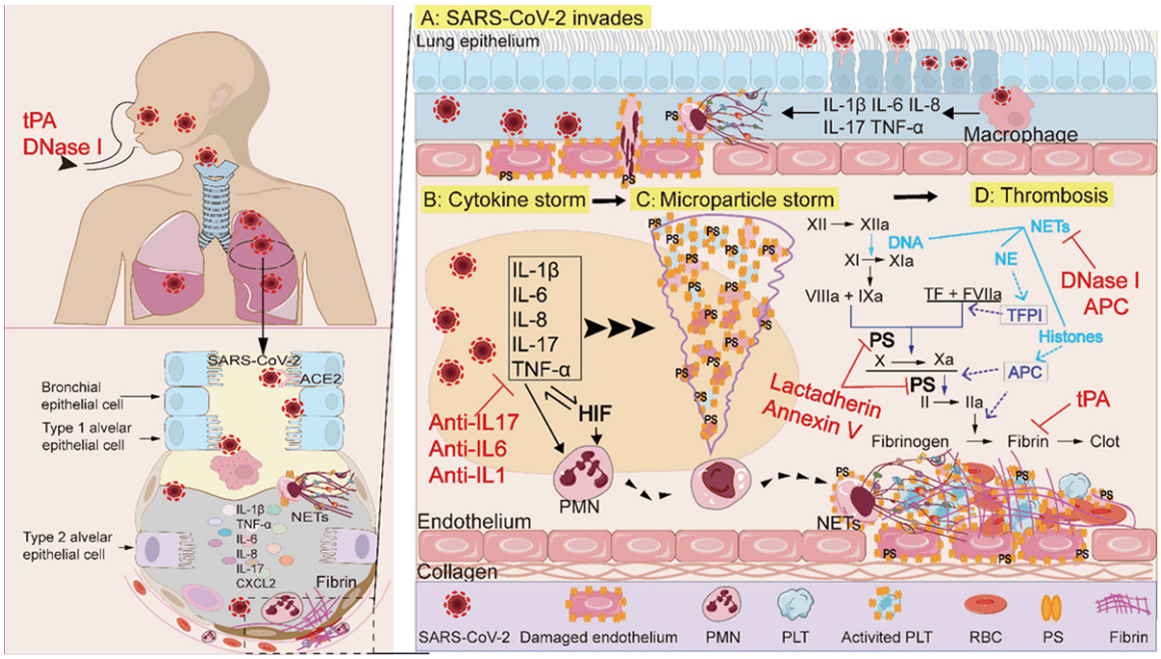

Figure 4. New targets for the treatment of thrombosis caused by COVID-19. Nebulized inhaled drugs improve pulmonary fibrosis, reduce lung tissue damage, and combine multiple targets to inhibit thrombosis, jointly reduce patient mortality and improve prognosis. Viruses damage the lung and promote the infiltration of inflammatory cells. Many neutrophils release NETs to further aggravate lung injury. The deposition of fibrin and decreased fibrinolytic capacity may lead to pulmonary fibrosis, which facilitates the deterioration of respiratory function. Inhaling tPA and NETs through atomization can reduce the degree of lung fibrosis and tissue damage, then improve respiratory function. Combining with inhibiting cytokines, PS on MPs and NETs, the activation of the coagulation pathway is suppressed, reduce the production of thrombin, and finally stop thrombosis. APC: activated protein C; NETs: neutrophil extracellular traps; PLT: Platelet; PMN: Polymorphonuclear neutrophil; PS: phosphatidylserine; tPA: tissue plasminogen activator.

increase oxygen saturation, it is still difficult to reverse. Accordingly, improving the hypoxic symptom of patients will reduce endothelial damage and decrease the risk of thrombosis.

In addition to the important role of blood cells in thrombosis, hemodynamics is also a mechanism of thrombosis formation in the Virchow's Triad [93, 94]. Bo Zhou reports a severe case of lower extremity deep venous thrombosis with arterial occlusion. Due to the endothelial injury of COVID-19 patients, if the activity is restricted, the shear rate of blood flow will be reduced. This condition will promote the adhesion of platelets, blood stasis, and thrombosis, especially the risk of venous thrombosis in the lower limbs is greatly increased [95]. Because severe COVID-19 patients are mainly bedridden, with limited voluntary activity, the blood flow shear rate is reduced. This condition will promote platelet adhesion, congestion and thrombosis, which will make the severe COVID-19 patients worse.

Therapy

According to the current situation of anticoagulant therapy for COVID-19 and our understanding of the mechanism of thrombosis, we propose our new target for anticoagulant therapy (**Figure 4**).

Cytokines inhibiter

As mentioned above, cytokine storm plays an important role for disease progression in COVID-19. The inflammatory response within 24 hours after admission may be related to the severity of the disease. So, suppression of inflammatory factors will delay disease progression [96]. Inhibition of IL-6 is a vigorous anti-inflammatory method because it accounts for a relatively large proportion. Tocilizumab, a commonly used inhibitor, has been shown to reduce inflammation levels and improve symptoms and prognosis in patients. In moderate COVID-19 patients, it was found that tocilizum-

ab was well tolerated and no adverse reactions [97]. Although tocilizumab can reduce inflammation, there are side effects with liver damage and gastrointestinal ulcers in critically ill patients [98, 99]. Kimmig LM et al. found inhibition of IL-6 may damage the clearance of the virus, secondary infection, leading to death in severe COVID-19 [100]. Therefore, it is important to carefully control the dosage and timing of use to maximize the benefits in severe and critically ill patients.

Anti-coagulant therapy

Heparin and LMWH, commonly anticoagulant drugs, bind to antithrombin III which mainly inhibits the activation of FX and the production of thrombin to achieve the purpose of anticoagulation [101]. Due to the high incidence of COVID-19 thrombosis, anticoagulation treatment is routinely administered for COVID-19. Once the diagnosis is confirmed, preventive anticoagulation is performed, and the dose of heparin is adjusted according to changes in the condition. However, its dosage is still controversial. Some patients have bleeding after medication, but compared with the serious consequences caused by thrombosis, the risk of bleeding can be temporarily ignored when used in a short time [32, 33]. Heparin also has anti-inflammatory effects through inhibiting the production of inflammatory factors, such as IL-6, IL-8, TNF-α [102, 103]. The heparin can counteract excessive inflammatory factors in the body and reduce inflammation and inflammation-promoted hypercoagulability in COVID-19. In addition, heparin can also protect the endothelium and reduce vascular leakage [101], playing a role in many ways to inhibit thrombosis. Therefore, the early use heparin will greatly reduce the occurrence of thrombosis.

Platelets counts varies in different COVID-19 studies. Studies reported thrombocytopenia in severe patients, although the decline was not serious, which is a sharp contrast to the disseminated intravascular coagulation [17, 104]. The reason for the difference may be the activation of platelets due to the excessive inflammatory environment [105]. Most anti-platelet drugs bind irreversibly to platelets, and the corresponding inhibitors have a relatively long half-life. In addition, the lack of relatively sensitive indicators can, quickly and effectively, detect changes in platelets [106]. Therefore, when using platelet inhibitors, relevant indicators should be closely monitored.

NETs inhibitors

Inhibiting the NETs production and hydrolyzing the structure and components may decrease the activation of the coagulation pathway (**Figure 4**). Studies have shown that NADPH oxidase and peptidyl dearginase 4 inhibitors can reduce NETs production [107, 108]. However, it may increase the risk of infection. DNase hydrolyzes the NETs structure, destroying the ability of NETs bind cells and inhibiting DNA activating endogenous clotting pathways [109]. Histone antibody is used to block the cytotoxic histones, prevent tissue damage, promote the activation of protein C, and improve the anticoagulation ability [110]. In addition, due to the large amount of granular protein attached to NETs, the accumulation of local tissues aggravates tissue damage. Many neutrophils infiltrated in the lung, fibrin deposition, inhibit lung respiratory function. Inhalation of tPA and DNase I by atomization may promote pulmonary fibrinolysis, improve respiratory function [111]. Therefore, targeted NETs may alleviate the hypercoagulable state of COVID-19 and reduce thrombosis.

Targeting PS

The central point of the reaction is PS on the membrane of MPs and activated cells. With the invasion of the virus, the immune system overreacts and releases numbers of cytokines, which will cause quantities of cell activation, mutilation, apoptosis and even necrosis, then cells will produce PS on the surface, forming a microparticle storm [112]. Because MP storm provides an anchor platform for coagulation factors. Previous studies have confirmed that annexin V and lactadherin can bind PS interrupt the cascade of coagulation [74, 90]. Therefore, we believe that inhibitors, annexin V and lactadherin, will significantly reduce the incidence of thrombosis in COVID-19 patients without increasing the risk of bleeding. In addition, our previous research on the anticoagulant effect of lactadherin was mainly confirmed in vitro experiments. Whether its anticoagulant effect in vivo is affected by the microenvironment is still unknown. It is necessary to study the in vivo anticoagulation with lactadherin,

and we believe that inhibitors, annexin V and lactadherin, will significantly reduce the incidence of thrombosis in COVID-19 patients without increasing the risk of bleeding.

Improving the state of hypoxia via early oxygen absorption

Hypoxia is a common manifestation of severe or critically ill COVID-19 patients [113, 114]. Some studies have suggested that asymptomatic hypoxia occurs in mild patients, and the body is in a state of "happy hypoxia" or "silent hypoxia". Chronic silent hypoxia may not only motivate circulating blood cells, but also cause chronic damage to endothelial cells, promote PS exposed, release microparticles, and promote thrombosis. Thus, we believe that low-flow oxygen inhalation will ameliorate the hypoxia state of the mild patient's internal environment. According to the progress of patients, it will be increased the oxygen intake, and performed mechanical ventilation if necessary, to minimize the cell activation and endothelial cell damage caused by hypoxia, and diminish the risk factors of thrombosis.

Conclusion

In summary, we described the pathophysiological and the thrombotic mechanism in COVID-19. We proposed that the microparticle storm and the PS may play a significant role in thrombosis for the first time. Although a variety of mechanisms causing thrombosis have been analyzed, the combined effect of multiple factors will promote PS exposed of cells and eventually activate the coagulation pathway. By targeting PS, it can inhibit the generation of thrombin, reduce the hypercoagulable state and decrease thrombosis, which may provide a new treatment direction for the current inherent model of anticoagulation programs in COVID-19 patients. We believe that the assessment of different patients, combined with multi-target inhibition of the activation of the coagulation pathway, the formulation of a reasonable anticoagulant program, to decrease the occurrence of thrombosis events, will greatly diminish the mortality of patients and improve the prognosis.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (81670128 and 81873433) and the Scientific Research Innovation Foundation of the First Hospital of Harbin Medical University (2020 M12).

Disclosure of conflict of interest

None.

Abbreviations

ACE2, Angiotensin-converting enzyme 2; APC, activated protein C; ARDS, Acute respiratory distress syndrome; cf-DNA, Cell free-DNA; COVID-19, Coronavirus disease-2019; CRP, C-reactive protein; ECs, endothelial cells; FVIIa, Activated factor VII; FXa, Activated factor X; GCSF, Granulocyte colony stimulating factor; GM-CSF, Granulocyte and macrophage colony stimulating factor; HIF, hypoxia-inducible factor; ICU, Intensive care unit; IL, Interleukin; LDH, Lactate dehydrogenase; LMWH, Low molecular weight heparin; LPS, Lipopolysaccharide; MERS, Middle East Respiratory syndrome; MPO, myeloperoxidase; MPs, Microparticles; NETs, Neutrophil extracellular traps; NF-κB, Nuclear factor kappa-B; PLT, Platelet; PMN, Polymorphonuclear neutrophil; PS, Phosphatidylserine; RBC, Red blood cell; SARS, Severe Acute respiratory syndrome; SARS-CoV-2, Severe acute respiratory syndrome coronavirus type 2; TF, Tissue factor; TFPI, tissue factor pathway inhibitor; TNF-α, Tumor necrosis factor-α; vWF, Von Willebrand factor.

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