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Commentary

# Immunothrombosis in severe COVID-19

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread all over the world immediately after the first patient infected with this virus was discovered in Wuhan, China, in December 2019. The World Health Organization has named the disease induced by SARS-CoV-2 as coronavirus disease 2019 (COVID-19). Although COVID-19 was initially recognized as an acute respiratory distress syndrome, recent studies have revealed that coagulation disorders and thrombotic events contribute to its high mortality [1, 2]. It has been demonstrated that SARS-CoV-2 infection induces vascular endothelial injury, resulting from coagulation [3]. However, the pathological feature of thrombus induced by SARS-CoV-2 remains incompletely understood.

In this issue of *EBioMedicine*, Leppkes and coworkers demonstrated that, in severe COVID-19 patients, neutrophils were increased in the blood, exhibiting a so-called low-density phenotype, were strongly activated, and decorated with platelets [4]. In addition, several serum or plasma markers, such as D-dimers, cell-free DNA, myeloperoxidase (MPO)- and neutrophil elastase (NE)-DNA complexes, and citrullinated histone H3 (citH3), were elevated in severe COVID-19 patients. Because these are degradation products of fibrin or neutrophil extracellular traps (NETs), an enhanced turnover of coagulation and NET formation appears to characterize severe COVID-19. Correspondingly, aggregated NETs were detected in the clots that occluded microvessels in the lungs and other organs of COVID-19 patients obtained by autopsy.

NETs—first described in 2004 as an important component of the immune system—are web-like DNA decorated with antimicrobial proteins, including MPO and NE, which are released from activated neutrophils [5]. Currently, two different forms of NETs, namely, lytic NETs with neutrophil death and non-lytic NETs without neutrophil death, are recognized [6]. Lytic NET formation is dependent on the production of reactive oxygen species (ROS) by the activation of NADPH oxidase. It has been suggested that ROS translocate peptidylarginine deiminase 4 (PAD4) from the cytoplasm to the nucleus. In the nucleus, PAD4 citrullinates the histone tail and alters the molecular conformation, resulting in a detachment of DNA from histones, which coil around histones. After the completion of lytic NET formation, NETs are digested by a plasma-derived DNase I. Therefore, the increase in serum or plasma levels of cell-free DNA, MPO- and NE-DNA complexes, and citH3 suggests an enhanced turnover of NET formation. Although NETs can trap and kill microbes, they are simultaneously harmful to the hosts. Up to now, relationships between excessive NETs and diverse diseases, including thrombosis and autoimmune diseases, such as systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody-associated vasculitis, and rheumatoid arthritis, have been demonstrated.

The mechanism of NET induction by SARS-CoV-2 is debatable. Neutrophils themselves do not express angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2. In contrast, vascular endothelial cells provide abundant ACE2 for SARS-CoV-2 next to alveolar epithelial cells in the lungs. Based on the loss of $CD31^+$ cells in the endothelium that were close to the aggregated NETs, Leppkes and coworkers suggested that the injury of vascular endothelial cells infected with SARS-CoV-2 could trigger neutrophil attraction and NET formation (Fig. 1). This is consistent with the concept of immunothrombosis [7]. However, another pathway via virus-mediated ROS production [8] may also be involved in NET formation after SARS-CoV-2 infection.

When pathogens injure vascular endothelial cells, coagulation is invoked, and simultaneously, damage-associated molecular patterns (DAMPs) are secreted from the damaged cells. Activated platelets and neutrophils attracted by DAMPs aggregate on the surface of damaged endothelial cells, and then neutrophils form lytic NETs. NETs further activate platelets and include fibrin, resulting in the formation of a robust immunothrombus. The physiological significance of immunothrombosis is regarded as protective of endothelial integrity, and for the containment and elimination of pathogens. It has not been determined whether immunothrombosis is a cause or result of severe COVID-19.

Leppkes and coworkers suggested that the prevention of excessive NET formation and aggregation could provide an approach to inhibit vascular occlusion and the development of severe COVID-19.

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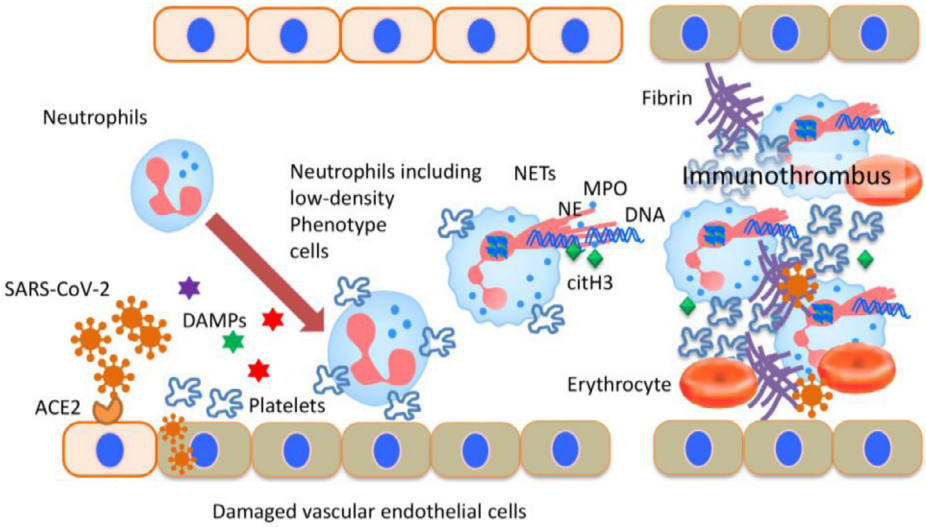


**Fig. 1.** Immunothrombosis induced by SARS-CoV-2

When SARS-CoV-2 injures vascular endothelial cells, coagulation is invoked, and simultaneously, DAMPs are secreted from the damaged cells. Activated platelets and neutrophils attracted by DAMPs aggregate on the surface of damaged endothelial cells, and then neutrophils form lytic NETs. NETs further activate platelets and include fibrin, resulting in the formation of a robust immunothrombus.

For this purpose, dexamethasone (a cell aggregation inhibitor) and PAD inhibitors (inhibitors of NET formation) may be considered. However, these drugs or agents may bring a risk of increased bloodstream infections. In the study of Leppkes et al., heparin accelerated NET degradation by DNase I. Moreover, previous studies have demonstrated that heparin can dismantle NETs and neutralize NET-derived histones, which are detrimental factors of NETs [9,10]. Although further studies are needed, this classical anticoagulant is a promising resource against severe COVID-19.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## References

[1] Al-Ani F, Chehade S, Lazo-Langner A. Thrombosis risk associated with COVID-19 infection. Scoping Rev Thromb Res 2020;192:152–60.
[2] Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. J Am Coll Cardiol 2020;75(23):2950–73.
[3] Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet 2020;395 (10234):1417–8.
[4] Leppkes M, Knopf J, Naschberger E, Lindemann A, Singh J, Herrmann I, et al. Vascular occlusion y neutrophil extracellular taps in COVID-19. EBioMedicine 2020.
[5] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303(5663):1532–5.
[6] Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 2018;18(2):134–47.
[7] Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. Respiration 2017;93(3):212–25.
[8] Muraro SP, De Souza GF, Gallo SW, Da Silva BK, De Oliveira SD, Vinolo MAR, et al. Respiratory syncytial virus induces the classical ROS-dependent NETosis through PAD-4 and necroptosis pathways activation. Sci Rep 2018;8 (1):14166.
[9] Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers Jr. DD, et al. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci U S A 2010;107 (36):15880–5.
[10] Kumar SV, Kulkarni OP, Mulay SR, Darisipudi MN, Romoli S, Thomasova D, et al. Neutrophil extracellular trap-related extracellular histones cause vascular necrosis in severe GN. J Am Soc Nephrol 2015;26(10):2399–413.