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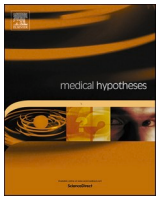

Is digital necrosis in COVID-19 caused by neutrophil extracellular traps: Potential therapeutic strategies

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ABSTRACT

Some of the COVID-19 patients present with ischemic lesions of their finger and toes. Standard anticoagulant therapy is usually unsuccessful for the treatment of this unique presentation of COVID-19. In this review current evidence is presented, which supports the hypothesis that these necrotic lesions are primarily related to the formation of neutrophil extracellular traps is blood vessels. Also, currently available and potential pharmacological methods of the management of this unique thrombotic complication are discussed. Drugs that possibly could be used in COVID-19 patients suffering from acute ischemia of distal parts of the extremities particularly comprise DNase I and DNase1L3, which could directly dissolve these extracellular webs that are mostly composed of DNA. However, at the moment, none of these enzymes are registered for an intravascular administration in humans. Lactoferrin and dipyridamole are other pharmaceutical agents that could potentially be used for the treatment of neutrophil extracellular traps-evoked digital ischemia. These agents exhibit prophylactic activity against excessive formation of these extracellular structures. Such an experimental treatment should probably be accompanied by standard antithrombotic management with heparin. Open-label and then randomized trials are needed to confirm feasibility, safety and efficacy of the above-suggested management of critically ill COVID-19 patients.

Background

Coronavirus disease-2019 (COVID-19) is primarily a respiratory system infection. However, some of these patients present with coexisting acute injuries to other organs, such as the heart and kidneys. Ischemic lesions in the upper or lower extremities due to arterial occlusions, usually affecting fingers and toes, represent another dangerous, although rare, complication associated with COVID-19. These ischemic lesions are usually seen in patients with severe clinical course of the disease. Most of them are difficult to manage and digital necroses either result in amputations, or contribute to final fatal outcome of the disease. Fig. 1.

Currently, pathogenesis of arterial thrombosis in COVID-19 patients remains elusive. However, clinical characteristics of these lesions, microscopic studies and findings in other organs affected by thrombosis in the settings of COVID-19 suggest that this pathology significantly differs from arterial thrombosis triggered by atherosclerosis.

Clinical characteristics of patients with COVID-19-associated digital necrosis

Until now, a number of case reports describing acute limb ischemia, usually manifesting as digital necroses, have been published [1–8]. Acute necrosis of fingers or toes, in some patients affecting also more proximal parts of the extremities, were typically seen in older individuals presenting with acute respiratory failure due to viral pneumonia. An average patients' age in these case reports (a total of 10 patients) was 66 years. Digital necrosis was more often seen in fingers (70%) than toes (30%); in one patient necrosis of the fingers was accompanied by a similar dry necrosis of the nose. Usually, these patients were managed with heparin, in some cases other anticoagulants also were used. A majority of these patients did not have a history of limb ischemia before COVID-19. Regarding these case reports, except for one patient who improved after anticoagulant therapy, pharmacological and surgical treatment were either unsuccessful, or the patients, due to their terminal clinical status, received palliative care only. Overall mortality rate among these patients was 80%.

There were also published two case series describing COVID-19

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patients presenting with acute limb ischemia. In the first of these papers, results of the treatment of 30 Peruvian patients were discussed [9]. Mean age of these patients was 60 years, only 53% of them presented with severe clinical course of COVID-19 (including 23% of asymptomatic individuals) and mortality rate in this group was only 23%. Ischemia was more often seen in the lower extremities. Besides, arterial occlusions were quite often present in the proximal arteries, in contrast to the patients described in case reports who usually developed distally-located arterial occlusions. A similar cohort of twenty COVID-19 patients presenting with acute limb ischemia comes from Italy (10). Similarly to the Peruvian report, in this study acute limb ischemia was predominantly seen in the arteries of lower extremities. Yet, Italian patients were older (75 years) and the mortality rate was higher (40%). Differences between the Peruvian and Italian cohorts were probably related to distinct demographic characteristics of these countries. Although probably some of the patients described in the Peruvian and Italian studies actually presented with typical acute limb ischemia, either of embolic or thrombotic etiology, yet the number of cases managed in the Italian centre was much higher if compared with a similar period in 2019; thus a majority of acute limb ischemia events in 2020 were likely to be associated with COVID-19 [10].

Macroscopic appearance of digital ischemia and very limited efficacy of anticoagulation in a majority patients described in case reports (and perhaps also in some of the patients from the Peruvian and Italian cohorts) suggest that pathomechanism responsible for these necrotic lesions is not primarily associated with classic activation of the coagulation cascade. In some of these patients autoantibodies typical for antiphospholipid syndrome are found. Still, such autoantibodies are not revealed in every patient. Nonetheless, a presence of the autoantibodies suggests that arterial occlusions in COVID-19 patients, at least in some cases, actually results from thrombotic processes evoked by immune reaction.

Hypothesis

In this paper current evidence suggesting that these ischemic lesions in COVID-19 patients are primarily caused by neutrophil extracellular traps (NETs) is presented. Also, potential pharmacological strategies aimed at the treatment of this unique type of thrombosis are discussed.

Neutrophil extracellular traps and COVID-19

There seem to be some similarities between severe antiphospholipid syndrome and diffuse coagulopathy associated with COVID-19. Of note, about 50% of COVID-19 patients test positive for antiphospholipid antibodies [11]. The already discovered pathomechanism responsible for vascular lesions seen in patients with antiphospholipid syndrome may shed some light at potential background of digital necrosis in COVID-19 patients. In antiphospholipid syndrome patients, arterial and venous thrombi are primarily evoked by the formation of NETs, which are web-like structures composed of DNA and proteins of the nuclear and granular origin. NETs are released by activated neutrophils that mobilize their chromatin, which—together with the nuclear proteins: histones—expands outside the neutrophils and decondensates. Formation of these web-like structures is an important part of the innate immune system, since NETs can ensnare and kill microorganisms. In addition, neutrophils release from their granules microbicide proteins and oxidant enzymes. However, because NETs activate platelets, an exaggerated formation of these extracellular webs can result in local thrombosis. In addition to the platelet activation, during formation of NETs, neutrophils release tissue factor, which initiates the coagulation cascade. Besides, these web-like structures bind coagulation factor XII and trigger its activation, and also induce inflammatory reaction in the vascular wall. Thus, a release of NETs is accompanied by an activation of coagulation, but the entire process is different from normal thrombosis. This unusual NET-associated thrombosis is sometimes referred to as immunothrombosis [12–14].

An unrestrained formation of NETs has already been found in the

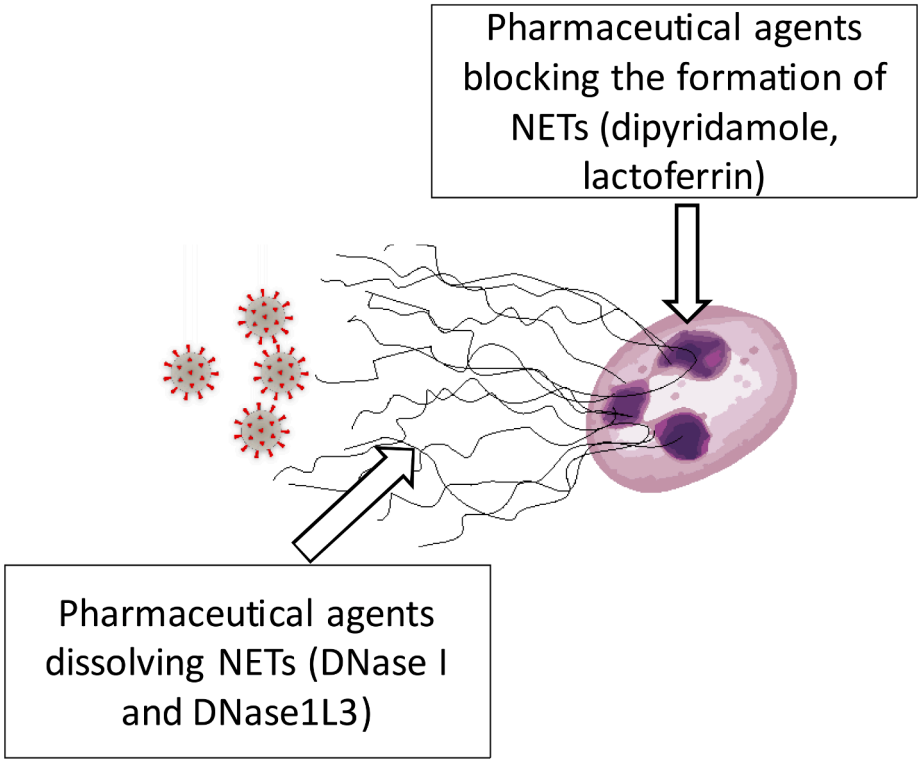

Fig. 1. Potential pharmaceutical strategies targeting NETs in COVID-19 patients.

settings of many diseases associated with inflammation and thrombosis, such as sepsis, antiphospholipid syndrome, acute respiratory distress syndrome and deep venous thrombosis. Interestingly, formation of NETs was also found in several autoimmune diseases, comprising rheumatoid arthritis, lupus and psoriasis, and even in some cancer patients [13–17]. There is already evidence demonstrating the formation of NETs in COVID-19 patients, primarily in pulmonary microcirculation [18–23]. It has also been shown that exaggerated NET development contributes to occlusion of pulmonary microvessels and to damage of the lungs in these patients. Of note, a higher neutrophil-to-lymphocyte ratio in the peripheral blood has already been found to be associated with severe clinical course of COVID-19 and an increased mortality rate [24–28].

Besides, there are some papers describing the occurrence of NETs in other blood vessels in COVID-19 patients [19,29]. In the case report of COVID-19 patient presenting with cerebral stroke resulting from thrombotic occlusion of the middle cerebral artery, immunohistochemical examination of the retrieved thrombus revealed an unusually small amount of fibrin. The thrombus mostly contained platelets, polymorphonuclear cells and also NETs [29]. In another study, describing acute coronary event in the COVID-19 patient, immunohistochemical analysis of thrombi removed from the coronary arteries demonstrated fibrin and polymorphonuclear cells, but no atherosclerotic plaques, which is characteristic for typical coronary artery disease [30]. Although, for the time being, no data regarding the composition of thrombi occluding the arteries in the upper and lower extremities in COVID-19 patients have been published, considering the fact that most of COVID-19 patients suffering from acute peripheral limb ischemia had no history of arterial occlusive disease, it is quite likely that a majority of such thrombotic materials develops in the setting of immunothrombosis. There has already been published article partially confirming the hypothesis that digital necrosis in COVID-19 patients is primarily related to the formation of NETs. In this case report, which described COVID-19 patient presenting with acute ischemia of his fingers and toes, biopsy taken from a livedoid lesion has revealed thrombotic occlusions of the cutaneous and subcutaneous arteries accompanied by neutrophilic infiltrate, thus suggesting pathological role for neutrophils in this unique COVID-19-associated pathology [31].

Mechanisms of the removal of NETs

For the time being, little is known about removal of NETs in vivo. Since these extracellular structures develop in the setting of a number of physiological and pathological conditions that very rarely result in severe vascular occlusions, it should be assumed that typically NET are efficiently cleared by natural mechanisms, as soon as these biological traps are no longer needed to fight against microorganisms. Since DNA is a backbone of NETs, fragmentation of DNA chains by DNase should be the principal mechanism responsible for NET clearance. The in vitro and in vivo experiments have already confirmed the role for DNases, primarily the DNase I and DNase1L3 (DNase γ) in this first step of NET removal. Subsequently, NET fragments are removed by phagocytic cells. There is already evidence, although limited, demonstrating that such a phagocytosis actually takes place. Yet, details of this process, especially in the settings of different pathologies, remain to be established [12]. Nonetheless, the process of NET removal is very different from that of a typical thrombus, which explains why anticoagulant, fibrinolytics and antiplatelet agents are of limited efficacy in the case of immunothrombosis. Although still very little is known about removal of NET remnants, it has already been revealed that this process is primarily executed by macrophages. These cells usually efficiently remove fragments of NETs, although quite slowly. However, when there is a large amount of NETs to be removed, macrophages can also release their DNA, similarly to the neutrophils, and form the so-called monocyte/macrophage extracellular traps (METs). If this occurs, there are even more DNA remnants to be removed and the entire process of NET and MET removal becomes highly ineffective [12].

Potential therapeutic strategies aimed at NET removal in the settings of COVID-19

Potential therapeutic strategies targeting an excessive formation of NETs in COVID-19 patients should focus at two pathomechanisms: blocking the formation of NETs and dissolving the already developed ones. The first process, at least theoretically, could be reduced by lactoferrin, but this protein is rather unlikely to be effective in severe cases [32]. Indeed, at the moment, several trials evaluating clinical efficacy of lactoferrin in COVID-19 are underway, but most of them include patients with mild clinical course of COVID-19. Another possible prophylaxis against NET formation regards dipyridamole. This pharmaceutical agent is characterized by an agonistic activity on the adenosine A2A receptor, which in turn reduces the release of NETs by neutrophils. There is already some evidence coming from animal experiments and a small clinical study from China encouraging such a prophylactic use of dipyridamole in COVID-19 patients [33,34]. Currently, three clinical trials evaluating efficacy of this antiplatelet agent for the treatment of COVID-19 have been registered and results of these studies are expected to be released in a few months.

The already developed NETs could theoretically be targeted with intravascular infusion of DNases [15]. In the case of COVID-19 patients presenting with acute respiratory distress that—in addition to the damage of the respiratory epithelium—is caused by occlusion of the pulmonary microvessels by NETs [35], it has already been suggested that inhalation of aerosol mist containing recombined human DNase I (Pulmozyme®, Genentech, South San Francisco, CA, USA) (an agent that is used for the treatment of cystic fibrosis) could improve results of the treatment [36,37]. At the moment, several clinical trials evaluating efficacy of an aerosolized recombinant DNase I for the treatment of COVID-19-associated respiratory failure are underway [38]. But in COVID-19 patients presenting with limb ischemia, DNase should be administered intravascularly. Currently, no pharmaceutical agent has been registered for this purpose. Theoretically, commercially available recombined human DNase I can be used, optimally in local intra-arterial infusion, since animal experiments demonstrated that after systemic intravenous administration this enzyme was rapidly cleared from the serum. Of note, preclinical human intravenous studies did not find toxic effect of this pharmaceutical agent and revealed the elimination half-life from the serum at the level of 3–4 h (data on pharmacokinetics of Pulmozyme® are available at: https://www.medicines.org.uk/emc/product/1112/smpc). There is, however, an important problem regarding efficacy of DNase I in the case of arterial immunothrombosis. DNase I cannot digest DNA that is already attached to the vascular wall glycocalyx, which typically occurs in vivo. This problem could theoretically be overcome if DNase1L3 were used instead or in combination with DNase I [33]. Still, for the time being, no commercially available DNase1L3 exists that could be used for such a treatment, although at least one biotechnology company is currently working on resolving this issue. Probably, such an experimental treatment of critically ill COVID-19 patients with DNases should be accompanied by standard antithrombotic management with heparin and possibly by prophylaxis against NET formation using lactoferrin and/or dipyridamole [39].

In conclusion, considering hypothetical pathomechanism of digital necrosis in COVID-19 patients, especially regarding possible role for NETs, it seems that other than standard antithrombotic management could be more effective. Open-label and then randomized trials are needed to confirm feasibility, safety and efficacy of the above-suggested pharmacotherapy in these critically ill COVID-19 patients.

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Consent statement/Ethical approval.

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Conflict of interest statement

There is no conflict of interest associated with the paper: “*Is digital*

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References

[1] Novara E, Molinaro E, Benedetti I, et al. Severe acute dried gangrene in COVID-19 infection: a case report. Eur Rev Med Pharmacol Sci 2020;24:5769–71.

[2] Shih L, Ferry AM, Gravina PR, et al. Acute digital necrosis in a patient with Raynaud's phenomenon and COVID-19 infection. Am Surg 2020; doi: 10.1177/0003134820979788.

[3] Wang JS, Pasieka HB, Petronic-Rosic V, et al. Digital gangrene as a sign of catastrophic coronavirus disease 2019-related microangiopathy. Plast Reconstr Surg Glob Open 2020;8:e3025.

[4] Del Giudice P, Boudoumi D, Le Guen B, Reverte M, Gutnecht J, Lacour JP, et al. Catastrophic acute bilateral lower limbs necrosis associated with COVID-19 as a likely consequence of both vasculitis and coagulopathy. J Eur Acad Dermatol Venereol 2020;34(11). https://doi.org/10.1111/jdv.v34.1110.1111/jdv.16763.

[5] Schultz K, Wolf JM. Digital ischemia in COVID-19 patients: case report. J Hand Surg Am 2020;45(6):518–22.

[6] Heald M, Fish J, Lurie F. Skin manifestations of COVID-19 resembling acute limb ischemia. J Vasc Surg Cases Innov Tech 2020;6(4):514–5.

[7] Scott B-A, Garg K, Johnson W, Al-Ajam M, Patalano P, Rotella V, et al. Upper extremity arterial thromboembolism in a coronavirus patient. A case report. SN Compr. Clin Med 2021;3(1):273–8. https://doi.org/10.1007/s42399-020-00694-2.

[8] Liu Y, Chen P, Mutar M, Hung M, Shao Z, Han Y, et al. Ischemic necrosis of lower extremity in COVID-19: A case report. J Atheroscler Thromb 2021;28(1):90–5.

[9] Sánchez JB, Cuipal Alcalde JD, Ramos Isidro R, Luna CZ, Cubas WS, Coaguila Charres A, et al. Acute limb ischemia in a Peruvian cohort infected by COVID-19. Ann Vasc Surg 2021;72:196–204. https://doi.org/10.1016/j.avsg.2020.12.005.

[10] Bellosta R, Luzzani L, Natalini G, Pegorer MA, Attisani L, Cossu LG, et al. Acute limb ischemia in patients with COVID-19 pneumonia. J Vasc Surg 2020;72(6):1864–72.

[11] Zuo Yu, Estes SK, Ali RA, Gandhi AA, Yalavarthi S, Shi H, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. Sci Transl Med 2020;12(570). https://doi.org/10.1126/scitranslmed.abd3876.

[12] Santocki M, Kolaczkowska E. On neutrophil extracellular trap (NET) removal: what we know thus far and why so little. Cells 2020;9(9):2079. https://doi.org/10.3390/cells9092079.

[13] Klopf J, Brostjan C, Eilenberg W, Neumayer C. Neutrophil extracellular traps and their implications in cardiovascular and inflammatory disease. Int J Mol Sci 2021;22(2):559. https://doi.org/10.3390/ijms22020559.

[14] Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: Cross talks between inflammation and coagulation. Respiration 2017;93(3):212–25.

[15] Ge L, Zhou X, Ji W-J, Lu R-Y, Zhang Y, Zhang Y-D, et al. Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: therapeutic potential of DNase-based reperfusion strategy. Am J Physiol Heart Circ Physiol 2015;308(5):H500–9.

[16] Laridan E, Denorme F, Desender L, François O, Andersson T, Deckmyn H, et al. Neutrophil extracellular traps in ischemic stroke thrombi. Ann Neurol 2017;82(2):223–32.

[17] Ducroux C, Di Meglio L, Loyau S, Delbosc S, Boisseau W, Deschildre C, et al. Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke. Stroke 2018;49(3):754–7.

[18] Yeh CH, de Wit K, Levy JH, Weitz JI, Vaezzadeh N, Liaw PC, et al. Hypercoagulability and coronavirus disease 2019-associated hypoxemic respiratory failure: Mechanisms and emerging management paradigms. J Trauma Acute Care Surg 2020;89(6):e177–81.

[19] Zuo Yu, Zuo M, Yalavarthi S, Gockman K, Madison JA, Shi H, et al. Neutrophil extracellular traps and thrombosis in COVID-19. J Thromb Thrombolysis 2021;51(2):446–53. https://doi.org/10.1007/s11239-020-02324-z.

[20] Hampton T. Autoantibodies may drive COVID-19 blood clots. JAMA 2021;325(5):425. https://doi.org/10.1001/jama.2020.25699.

[21] Iba T, Warkentin TE, Thachil J, Levi M, Levy JH. Proposal of the definition for COVID-19-associated coagulopathy. J Clin Med 2021;10(2):191. https://doi.org/10.3390/jcm10020191.

[22] Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med 2020;217:e20200652.

[23] Singhania N, Bansal S, Nimmatoori DP, Ejaz AA, McCullough PA, Singhania G. Current overview on hypercoagulability in COVID-19. Am J Cardiovasc Drugs 2020;20(5):393–403. https://doi.org/10.1007/s40256-020-00431-z.

[24] Asghar A, Saqib U, Aijaz S, et al. Utility of neutrophil-to-lymphocyte ratio, platelets-to-lymphocyte ratio and call score for prognosis assessment in COVID-19 patients. Pak Armed Forces Med J 2020;70:S590–6.

[25] Ciccullo A, Borghetti A, Zileri Dal Verme L, Tosoni A, Lombardi F, Garcovich M, et al. Neutrophil-to-lymphocyte ratio and clinical outcome in COVID-19: a report from the Italian front line. Int J Antimicrob Agents 2020;56(2):106017. https://doi.org/10.1016/j.ijantimicag.2020.106017.

[26] Pimentel GD, Dela Vega MCM, Laviano A. High neutrophil to lymphocyte ratio as a prognostic marker in COVID-19 patients. Clin Nutr ESPEN 2020;40:101–2.

[27] Haghjooy Javanmard S, Vaseghi G, Manteghinejad A, Nasirian M. Neutrophil-to-lymphocyte ratio as a potential biomarker for disease severity in COVID-19 patients. J Glob Antimicrob Resist 2020;22:862–3.

[28] Vasmehjani AA, Mardani R, Azadani HN, Zali,, et al. Neutrophil to lymphocyte ratio and C-reactive protein level as prognostic markers in mild versus severe COVID-19 patients. Gastroenterol Hepatol Bed Bench 2020;13:361–6.

[29] Boeckh-Behrens T, Golkowski D, Ikenberg B, et al. COVID-19-associated large vessel stroke in a 28-year-old patient. NETs and platelets possible key players in acute thrombus formation. Clin Neuroradiol 2021. https://doi.org/10.1007/s00062-020-00992-1.

[30] Blasco Ana, Coronado María-José, Hernández-Terciado Fernando, Martín Paloma, Royuela Ana, Ramil Elvira, et al. Assessment of neutrophil extracellular traps in coronary thrombus of a case series of patients with COVID-19 and myocardial infarction. JAMA Cardiol 2021;6(4):469. https://doi.org/10.1001/jamacardio.2020.7308.

[31] Llamas-Velasco M, Muñoz-Hernández P, Lázaro-González J, et al. Thrombotic occlusive vasculopathy in a skin biopsy from a livedoid lesion of a patient with COVID-19. D Br J Dermatol 2020;183:591–3.

[32] Okubo Koshu, Kamiya Mako, Urano Yasuteru, Nishi Hiroshi, Herter Jan M, Mayadas Tanya, et al. Lactoferrin suppresses neutrophil extracellular traps release in inflammation. EBioMedicine 2016;10:204–15.

[33] Liu X, Li Z, Liu S, et al. Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19. Acta Pharm Sin B 2020;10:1205e1215.

[34] Ali Ramadan A, Gandhi Alex A, Meng He, Yalavarthi Srilakshmi, Vreede Andrew P, Estes Shanea K, et al. Adenosine receptor agonism protects against NETosis and thrombosis in antiphospholipid syndrome. Nat Commun 2019;10(1). https://doi.org/10.1038/s41467-019-09801-x.

[35] Leppkes Moritz, Knopf Jasmin, Naschberger Elisabeth, Lindemann Aylin, Singh Jeeshan, Herrmann Irmgard, et al. Vascular occlusion by neutrophil extracellular traps in COVID-19. EBioMedicine 2020;58:102925. https://doi.org/10.1016/j.ebiom.2020.102925.

[36] Okur HK, Yalcin K, Tastan C, et al. Preliminary report of in vitro and in vivo effectiveness of dornase alfa on SARS-CoV-2 infection. New Microbe New Infect 2020;37:100756.

[37] Lee Yun Young, Park Hee Ho, Park Wooram, Kim Hyelim, Jang Jong Geol, Hong Kyung Soo, et al. Long-acting nanoparticulate DNase-1 for effective suppression of SARS-CoV-2-mediated neutrophil activities and cytokine storm. Biomaterials 2021;267:120389. https://doi.org/10.1016/j.biomaterials.2020.120389.

[38] Earhart AP, Holliday ZM, Hofmann HV, Schrum AG. Consideration of dornase alfa for the treatment of severe COVID-19 acute respiratory distress syndrome. New Microbes New Infect 2020;35:100689. https://doi.org/10.1016/j.nmni.2020.100689.

[39] Bikdeli Behnood, Madhavan Mahesh V, Gupta Aakriti, Jimenez David, Burton John R, Der Nigoghossian Caroline, et al. Pharmacological agents targeting thromboinflammation in COVID-19: review and implications for future research. Thromb Haemost 2020;120(07):1004–24.