

Perspective

SARS-CoV2 may evade innate immune response, causing uncontrolled neutrophil extracellular traps formation and multi-organ failure

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We demonstrate that the general clinical conditions, risk factors and numerous pathological and biological features of COVID-19 are analogous with various disorders caused by the uncontrolled formation of neutrophil extracellular traps and their by-products. Given the rapid evolution of this disease's symptoms and its lethality, we hypothesize that SARS-CoV2 evades innate immune response causing COVID-19 progresses under just such an amplifier loop, leading to a massive, uncontrolled inflammation process. This work allows us to propose new strategies for treating the pandemic.

December 2019 saw the emergence of the Severe acute respiratory syndrome – Coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease-2019 (COVID-19) [1]. Several clinical syndromes associated with SARS-CoV2 are described: asymptomatic forms, uncomplicated disease, non-severe pneumonia and severe pneumonia, acute respiratory distress syndrome (ARDS), a life-threatening respiratory failure, and also sepsis and septic shock with multivisceral failure syndrome. Patients with COVID-19 display polymorphic manifestations including clinical features like fever, nonproductive cough, dyspnea, myalgia, fatigue, with paraclinical characteristics like normal or decreased leukocyte counts, and radiographic evidence of pneumonia. Accumulating evidence reveals that an excessive and uncontrolled release of pro-inflammatory cytokines, called cytokine storm, occurs frequently in severe cases. This cytokine storm leads to ARDS, multiple organ damage and even death. The COVID-19 cytokine storm is also clearly characterized in critically ill patients by substantial impairment of the host immune system and, in particular, the innate immune response [2].

Neutrophils play an important role as the first line of innate immune defense. One of their functions known as neutrophil extracellular traps (NETs) was discovered in 2004 [3–5]. These are extensive structures released extracellularly from activated neutrophils in response to infection. They are composed of granular protein assembled on a scaffold of released chromatin. These structures impede the dissemination of microorganisms in blood by trapping them mechanically, and by exploiting coagulant function to segregate them within the circulation [6]. NET components (DNA, histones, granule proteins) also contribute to the triggering of an inflammatory process [3–6].

NET function, however, can be considered a ‘double-edged sword’ [7]. On one hand, as an innate immune response, NET formation is an efficient strategy for neutralizing invasive microorganisms. On the other hand, NET can be harmful to the host, in that its exposed by-products are toxic to endothelial cells and parenchymal tissue. Unbalanced NET formation and neutrophil activation may therefore play a significant role in the pathogenesis of numerous non-autoimmune pathologies, such as thrombosis, cystic fibrosis, sepsis, transfusion-related acute lung injury, severe obesity, gouty arthritis, pre-eclampsia or kidney diseases; and in the pathogenesis of autoimmune diseases such as lupus, Type 1 diabetes, vasculitis or rare conditions affecting small blood vessels, particularly those of the lungs, skin and kidneys [5] (Table 1A) [8–20].

Received: 28 April 2020
 Revised: 02 June 2020
 Accepted: 04 June 2020

Accepted Manuscript online:
 04 June 2020
 Version of Record published:
 16 June 2020

Table 1 Physiopathological link between COVID-19 and dysregulation of NET formation

A	B
Common pathological conditions or biological features	COVID-19 comorbidities/host risk factors
Overall	Non-auto-immuno
Complex disease	Pulmonary diseases*
Inflammatory disease	Cerebrovascular disease*
Multi-organ damage	Kidney diseases*
Pathologies	Cardiovascular disease*
Respiratory failure	Hypertension*
ARDS	Obesity*
Heart failure	Chronic inflammation disease
Acute cardiac injury	Disseminated intravascular coagulation
Sepsis	Sepsis
Type 1 Diabetes sensitization	Sickle cell disease
Kidney diseases	Auto-immune
Inflammatory bowel disease	Type 1 diabetes
Chronic inflammation disease	<i>Rheumatoid arthritis</i>
<i>Rheumatoid arthritis</i>	
<i>Neuropathy</i>	
<i>Gouty arthritis sensitization</i>	
Vascular and coagulation consequences	
Disseminated intravascular coagulation	
Endothelium damage	
Systemic vascular permeability	
Prothrombotic	
Abnormality of coagulation function	
Biological features	
High level neutrophils	
High level interferon	
High level C reactive protein	
High level proinflammatory cytokines	
Elevated presence of fibrinogen	
High level antiphospholipid antibodies	

A: Common physiopathological conditions or biological features of COVID-19 and diverse pathologies caused by NETosis dysregulation. *Italic:* pathologies found in a minority of cases. **B:** COVID-19 comorbidities/host risk factors. *Italic:* suggested comorbidity observed as trends. No italic: reported comorbidity.

All COVID-19 comorbidities except cerebrovascular disease and immunodeficiency, are NETosis relevant diseases. Data updated up to April 22, 2020. *Comorbidity as independent risk factors as reported by McMichael et al. [27].

There are various approaches to controlling NET formation in the context of viral infection. Naturally occurring deoxyribonuclease I (DNase-1) digests extracellular chromatin and NETs [10–14]. Low level bioactivity of endogenous DNase-1 may lead to a dysregulation of NETs, thus causing autoimmune diseases and other inflammatory disorders. DNase-1 is the only NET-targeting molecule already in use in clinical practice, as it is used to treat both cystic fibrosis in order to improve lung function and reduce infectious exacerbations, and virus-associated bronchiolitis [15,16]. However, the fact that DNase-1 dismantles the NET structure without degrading the whole protein components of NETs, and indicates that it is less effective in abrogating a NET-triggered inflammatory response. The latter can be targeted with using histone-blocking antibodies [9]. As regards neutrophil–platelet interactions, aspirin treatment decreases NET formation in the lung microcirculation and plasma, and also decreases the deposition of platelets with neutrophils on lung vascular walls [9]. Very different structural classes of molecules can inhibit the potent neutrophil stimulus for the release of NETs by platelet activation of endosomal toll-like receptors (TLRs) [10]. Such approaches include anti-CLEC (C-type Lectin-like receptors) [12] and especially a bispecific anti-CLEC5A/TLR2 monoclonal antibody [13]. Hydroxychloroquine, a broadly anti-malarial and anti-inflammatory drug, shows TLR-pathway blockage capacity [21]. Note, Zuo et al. [22] recently showed that hydroxychloroquine treatment in COVID-19 does not affect the level of NETs in the serum of hospitalized patients showing either mild or severe forms of the disease. The use of biologics to block cytokines is now widespread, as in the use of newer, small molecule drugs such as ‘Jak inhibitors’ [23], or anti-interleukin 6 (IL-6) approaches to block neutrophil function [24]. Self-DNA re-entry may be recognized by

TLR DNA sensors as damage-associated molecular patterns (DAMPs); the observation of IL-26 as a ‘cargo’ molecule for DNA, therefore, suggests the possibility of targeting IL-26, as part of a strategy to reduce inflammatory response [25].

In all of this, we are flagging the analogous biological and physiological features of COVID-19 infection [1,2,26] (Table 1A) and the detrimental amplification loop between inflammation and tissue damage induced by NETosis dysregulation (Table 1A). Widely described, both are complex diseases that result in inflammatory processes and multi-organ damages. More specifically, both are associated with an abnormality of coagulation factors, prothrombotic activity and with cytotoxicity toward endothelial and epithelial cells, leading in particular to systemic vascular permeability [2,25]. As a result of this, vasculitis, myocardial infarction, hemorrhage or systemic side effects on the blood supply and on the functions of multiple organs are observed in both disorders (Table 1A). With respect to biologics, their effects include overconcentration of neutrophils in lung vascularization and high levels of interferon, C reactive protein, lactate dehydrogenases, proinflammatory cytokines and high amount of circulating fibrinogen. Accordingly, both may lead to failure of respiration function to the extent of ARDS, and also thrombosis, sepsis, acute cardiac injury and heart failure [1,2,23,26–29] (Table 1B).

High levels of circulating NETs (and related increased amounts of circulating DNA and histones) are detected in patients with viral infections such as hantavirus or human immunodeficiency virus (HIV). NETs and neutrophils are also involved in the pathologies of chikungunya virus, simian immunodeficiency virus, influenza, parvovirus, rhinovirus and influenza-associated pneumonia [19,30,31].

Although most people with COVID-19 have mild to moderate symptoms, the disease can cause severe medical complications, and in some individuals leads to death. Older adults or people with existing chronic medical conditions are at greater risk of evolving toward a serious form of COVID-19 [1,2,27]. As recently reported, hypertension, obesity, diabetes mellitus and cardiac, renal or pulmonary diseases were found to be the most common chronic underlying health conditions in old residents with COVID-19 in a long-term care facility [28]. Other comorbidities were recently reported: inflammatory bowel disease, disseminated intravascular coagulation, sepsis, rheumatoid arthritis, and chronic inflammation and Sickle cell diseases [9,32–35] (Table 1B). All these clinical conditions correlate with the pathologies resulting from NETosis dysregulation [36–46] (Table 1A).

We were amongst the very first to report COVID-19 pathogenesis in light of NETs formation [28], at a moment which saw several groups independently and concurrently reach similar conclusions to our own [22,47,48]. Here, we showed the risk factor correlation, and that most of COVID-19’s pathological and biological features are analogous with the deleterious effects of NET dysregulation (Table 1A). Acknowledging, of course, that correlation does not equal causation, we are nonetheless confident in our hypothesis that COVID-19 progresses under an amplifier loop, leading to a massive, uncontrolled inflammation process, so called cytokine storm, which is due in part at least to unbalanced NET formation. We postulate that SARS-CoV2 induces a disproportionate virus-induced NET release, and that this plays a key role in the COVID-19 pathogenesis. Further to this, we speculate that these patients may have pathogenic host factors that allow SARS-CoV2 to find ways of evading the innate immune response, and that this may in turn generate chronic NET auto-stimulation, whose impact is that of an autoimmune-like disease. It should be noted that a recent experimental work clearly supports our account of the link between COVID-19 and NETosis [22].

NETs appear to involve several mechanisms, as evidenced by the variety of deleterious effects of NETs and by-products following SARS-CoV2 infection. First, overformation of NETs may trigger thrombosis by attracting platelets and fibrinogen [5,7,16,49]. Extracellular DNA of mitochondrial or nuclear origin may stimulate the inflammatory response that participates in the ‘cytokine storm’ in an auto-inflammatory process [4,11,19,44,45]. Circulating histones may contribute to this process [4,11]. Granulated proteases such as elastase may facilitate the virus cell entry by enabling Spike protein cleavage [50], and by interfering with epithelial Na⁺ transport leading to inefficient mucociliary clearance [51]. In addition, a high concentration of local or circulating proteases is toxic to endothelial and epithelial cells leading to serious multi-organ tissue damage, in particular to vascular walls [52]. Note, no formation of NETs by any other coronavirus was previously observed.

While neutrophils are the main starting point for extracellular and circulating DNA release, an effective strategy may be to target NETs rather than neutrophils themselves. Considering the severe impact of the COVID-19 pandemic on public health, the clinical imperative now should be to implement combination therapy with drugs currently used on patients or in the final stages of clinical development. These drugs should include Remdesivir (GS-5734), a Lopinavir/Ritonavir association combined or not (with or without interferon β -1a), hydroxychloroquine, anti-IL-6, Jakinibs or intravenous immunoglobulins [2,22]. Note, these drugs did not show major effects in clinical trials. We propose that, in the short term, DNase-1 treatment should be evaluated in clinical trials [53,54], with or without

associated drugs. We also propose, in the longer term, a significant increase in research on the development of TLR and CLEC inhibitors, and of anti-IL26 therapies.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

A.R. Thierry is supported by INSERM. This work was funded by the ‘SIRIC Montpellier Cancer Grant INCa.Inserm.DGOS.12553’.

Acknowledgements

The authors thank S. Dejasse, C. Mc Carthy, J. Lopez and RC Gallo.

Abbreviations

ARDS, acute respiratory distress syndrome; CLEC, C-type lectin-like receptor; COVID-19, coronavirus disease-2019; DAMP, damage-associated molecular pattern; DNase-1, deoxyribonuclease I; HIV, human immunodeficiency virus; IL-6, interleukin 6; NET, neutrophil extracellular trap; TLR, toll-like receptor.

References

- Wu, F. et al. (2020) A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265–269, <https://doi.org/10.1038/s41586-020-2008-3>
- Li, X., Geng, M., Peng, Y., Meng, L. and Lu, S. (2020) Molecular immune pathogenesis and diagnosis of COVID-19. *J. Pharmaceut. Analysis* S2095177920302045
- Brinkmann, V. (2018) Neutrophil Extracellular Traps in the Second Decade. *J. Innate Immun.* **10**, 414–421, <https://doi.org/10.1159/000489829>
- Elkon, K.B. (2018) Review: Cell Death, Nucleic Acids, and Immunity: Inflammation Beyond the Grave. *Arthritis Rheumatol.* **70**, 805–816, <https://doi.org/10.1002/art.40452>
- Pertiwi, K. et al. (2018) Neutrophil Extracellular Traps Participate in All Different Types of Thrombotic and Haemorrhagic Complications of Coronary Atherosclerosis. *Thromb. Haemost.* **118**, 1078–1087
- Granger, V., de Chaisemartin, L. and Chollet-Martin, S. (2014) La pêche miraculeuse des filets du neutrophile. *Med. Sci. (Paris)* **30**, 544–549, <https://doi.org/10.1051/medsci/20143005018>
- Stiel, L., Mayeur-Rousse, C., Helms, J., Meziani, F. and Mauvieux, L. (2019) First visualization of circulating neutrophil extracellular traps using cell fluorescence during human septic shock-induced disseminated intravascular coagulation. *Thrombosis Res.* **183**, 153–158, <https://doi.org/10.1016/j.thromres.2019.09.036>
- Euler, M. and Hoffmann, M.H. (2019) The double-edged role of neutrophil extracellular traps in inflammation. *Biochem. Soc. Trans.* BST20190629, <https://doi.org/10.1042/BST20190629>
- Hirose, T. et al. (2014) Presence of Neutrophil Extracellular Traps and Citrullinated Histone H3 in the Bloodstream of Critically Ill Patients. *PLoS ONE* **9**, e111755, <https://doi.org/10.1371/journal.pone.0111755>
- Caudrillier, A. et al. (2012) Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J. Clin. Invest.* **122**, 2661–2671, <https://doi.org/10.1172/JCI61303>
- Cheng, O.Z. and Palaniyar, N. (2013) NET balancing: a problem in inflammatory lung diseases. *Front. Immunol.* **4**, <https://doi.org/10.3389/fimmu.2013.00001>
- Lamphier, M. et al. (2014) Novel Small Molecule Inhibitors of TLR7 and TLR9: Mechanism of Action and Efficacy In Vivo. *Mol. Pharmacol.* **85**, 429–440, <https://doi.org/10.1124/mol.113.089821>
- Sung, P.-S. and Hsieh, S.-L. (2019) CLEC2 and CLEC5A: Pathogenic Host Factors in Acute Viral Infections. *Front. Immunol.* **10**, 2867, <https://doi.org/10.3389/fimmu.2019.02867>
- Zamudio-Cuevas, Y. et al. (2016) Monosodium urate crystals induce oxidative stress in human synoviocytes. *Arthritis Res. Ther.* **18**, 117, <https://doi.org/10.1186/s13075-016-1012-3>
- Nakazawa, D., Marschner, J.A., Platen, L. and Anders, H.-J. (2018) Extracellular traps in kidney disease. *Kidney Int.* **94**, 1087–1098, <https://doi.org/10.1016/j.kint.2018.08.035>
- Fuchs, T.A., Kremer Hovinga, J.A., Schatzberg, D., Wagner, D.D. and Lämmle, B. (2012) Circulating DNA and myeloperoxidase indicate disease activity in patients with thrombotic microangiopathies. *Blood* **120**, 1157–1164, <https://doi.org/10.1182/blood-2012-02-412197>
- Shida, H. et al. (2018) Anti-neutrophil extracellular trap antibody in a patient with relapse of anti-neutrophil cytoplasmic antibody-associated vasculitis: a case report. *BMC Nephrol.* **19**, 145, <https://doi.org/10.1186/s12882-018-0953-y>
- Bach, M. et al. (2020) A Neutrophil Activation Biomarker Panel in Prognosis and Monitoring of Patients With Rheumatoid Arthritis. *Arthritis Rheumatol.* **72**, 47–56, <https://doi.org/10.1002/art.41062>
- Schönrich, G. and Raftery, M.J. (2016) Neutrophil Extracellular Traps Go Viral. *Front. Immunol.* **7**, <https://doi.org/10.3389/fimmu.2016.00366>
- Yousefi, S. et al. (2019) Untangling “NETosis” from NETs. *Eur. J. Immunol.* **49**, 221–227, <https://doi.org/10.1002/eji.201747053>

- 21 Devaux, C.A., Rolain, J.-M., Colson, P. and Raoult, D. (2020) New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *Int. J. Antimicrobial Agents* 105938, <https://doi.org/10.1016/j.ijantimicag.2020.105938>
- 22 Zuo, Y., Yalavarthi, S., Shi, H. et al. (2020) Neutrophil extracellular traps in COVID-19. *JCI Insight*, Published online April 24, <https://doi.org/10.1172/jci.insight.138999>
- 23 Mehta, P. et al. (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet North Am. Ed.* **395**, 1033–1034, [https://doi.org/10.1016/S0140-6736\(20\)30628-0](https://doi.org/10.1016/S0140-6736(20)30628-0)
- 24 Wright, H.L., Cross, A.L., Edwards, S.W. and Moots, R.J. (2014) Effects of IL-6 and IL-6 blockade on neutrophil function in vitro and in vivo. *Rheumatology* **53**, 1321–1331, <https://doi.org/10.1093/rheumatology/keu035>
- 25 Poli, C. et al. (2017) IL-26 Confers Proinflammatory Properties to Extracellular DNA. *J. Immunol.* **198**, 3650–3661, <https://doi.org/10.4049/jimmunol.1600594>
- 26 Zhang, W. et al. (2020) The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clin. Immunol.* **214**, 108393, <https://doi.org/10.1016/j.clim.2020.108393>
- 27 Li, X. et al. (2020) Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J. Allergy Clin. Immunol.* S0091674920304954
- 28 McMichael, T.M. et al. (2020) Epidemiology of Covid-19 in a Long-Term Care Facility in King County, Washington. *N. Engl. J. Med.* NEJMoa2005412
- 29 Thierry, A.R. and Roch, B. (2020) NETs By-products and Extracellular DNA May Play a Key Role in COVID-19 Pathogenesis: Incidence on Patient Monitoring and Therapy. *Preprints* 2020040238
- 30 Sivanandham, R. et al. (2018) Neutrophil extracellular trap production contributes to pathogenesis in SIV-infected nonhuman primates. *J. Clin. Investigation* **128**, 5178–5183, <https://doi.org/10.1172/JCI99420>
- 31 Hiroki, C.H. et al. (2020) Neutrophil Extracellular Traps Effectively Control Acute Chikungunya Virus Infection. *Front. Immunol.* **10**, 3108
- 32 Hussain, F.A., Njoku, F.U., Saraf, S.L., Molokie, R.E., Gordeuk, V.R. and Han, J. (2020) COVID-19 Infection in Patients with Sickle Cell Disease. *Br. J. Haematol.* bjh.16734, Published online April 21, <https://doi.org/10.1111/bjh.16734>
- 33 Danese, S., Roda, G. and Peyrin-Biroulet, L. (2020) Evolving therapeutic goals in ulcerative colitis: towards disease clearance. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 1–2, <https://doi.org/10.1038/s41575-019-0211-1>
- 34 Favalli, E.G., Ingegnoli, F., De Lucia, O., Cincinelli, G., Cimaz, R. and Caporali, R. (2020) COVID-19 infection and rheumatoid arthritis: Faraway, so close!. *Autoimmun Rev.* **19**, 102523, <https://doi.org/10.1016/j.autrev.2020.102523>
- 35 Raoult, D., Zumla, A., Locatelli, F., Ippolito, G. and Kroemer, G. (2020) Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. *Cell Stress* **4**, 66–75, <https://doi.org/10.15698/cst2020.04.216>
- 36 Grommes, J. and Soehnlein, O. (2011) Contribution of Neutrophils to Acute Lung Injury. *Mol. Med.* **17**, 293–307, <https://doi.org/10.2119/molmed.2010.00138>
- 37 Fousert, E., Toes, R. and Desai, J. (2020) Neutrophil Extracellular Traps (NETs) Take the Central Stage in Driving Autoimmune Responses. *Cells* **9**, 915, <https://doi.org/10.3390/cells9040915>
- 38 D'Abbondanza, M., Martorelli, E.E., Ricci, M.A. et al. (2019) Increased plasmatic NETs by-products in patients in severe obesity. *Sci. Rep.* **9**, 14678, <https://doi.org/10.1038/s41598-019-51220-x>
- 39 Schimmel, M., Nur, E., Biemond, B.J. et al. (2013) Nucleosomes and neutrophil activation in sickle cell disease painful crisis. *Haematologica* **98**, 1797–1803, <https://doi.org/10.3324/haematol.2013.088021>
- 40 Bonaventura, A., Vecchié, A., Abbate, A. and Montecucco, F. (2020) Neutrophil Extracellular Traps and Cardiovascular Diseases: An Update. *Cells* **9**, 231, <https://doi.org/10.3390/cells9010231>
- 41 Vijay-Kumar, M., Saha, P., Yeoh, B.S. et al. (2019) Abstract P1126: Neutrophil Extracellular Traps: New Players in Hypertension. *Hypertension* **74**, <https://doi.org/10.1161/hyp.74.suppl.1.P1126>
- 42 Tang, N., Li, D., Wang, X. and Sun, Z. (2020) Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* **18**, 844–847, <https://doi.org/10.1111/jth.14768>
- 43 Tan, K.S., Lim, R.L., Liu, J. et al. (2020) Respiratory Viral Infections in Exacerbation of Chronic Airway Inflammatory Diseases: Novel Mechanisms and Insights From the Upper Airway Epithelium. *Front. Cell Dev. Biol.* **8**, 99, <https://doi.org/10.3389/fcell.2020.00099>
- 44 Angelidou, I., Chrysanthopoulou, A., Mitsios, A. et al. (2018) REDD1/Autophagy Pathway Is Associated with Neutrophil-Driven IL-1 β Inflammatory Response in Active Ulcerative Colitis. *J. Immunol.* **200**, 3950–3961, <https://doi.org/10.4049/jimmunol.1701643>
- 45 Menegazzo, L., Cicilioti, S., Poncina, N. et al. (2015) NETosis is induced by high glucose and associated with type 2 diabetes. *Acta Diabetol.* **52**, 497–503, <https://doi.org/10.1007/s00592-014-0676-x>
- 46 Rohrbach, A.S., Hemmers, S., Arandjelovic, S., Corr, M. and Mowen, K.A. (2012) PAD4 is not essential for disease in the K/BxN murine autoantibody-mediated model of arthritis. *Arthritis Res. Ther.* **14**, R104, <https://doi.org/10.1186/ar3829>
- 47 Golonka, R.M., Saha, P., Yeoh, B.S. et al. (2020) Harnessing Innate Immunity to Eliminate SARS-CoV-2 and Ameliorate COVID-19 Disease. *Physiol. Genomics*. physiolgenomics.00033.2020, Published online April 10
- 48 Barnes, B.J., Adrover, J.M., Baxter-Stoltzfus, A. et al. (2020) Targeting potential drivers of COVID-19: Neutrophil extracellular traps. *J. Exp. Med.* **217**, e20200652, <https://doi.org/10.1084/jem.20200652>
- 49 Pfeiler, S., Stark, K., Massberg, S. and Engelmann, B. (2017) Propagation of thrombosis by neutrophils and extracellular nucleosome networks. *Haematologica* **102**, 206–213, <https://doi.org/10.3324/haematol.2016.142471>
- 50 Belouzard, S., Madu, I. and Whittaker, G.R. (2010) Elastase-mediated Activation of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein at Discrete Sites within the S2 Domain. *J. Biol. Chem.* **285**, 22758–22763, <https://doi.org/10.1074/jbc.M110.103275>
- 51 Musante, I., Scudieri, P., Venturini, A. et al. (2019) Peripheral localization of the epithelial sodium channel in the apical membrane of bronchial epithelial cells. *Exp. Physiol.* **104**, 866–875, <https://doi.org/10.1113/EP087590>

- 52 Barbosa da Cruz, D., Helms, J., Aquino, L.R. et al. (2019) DNA-bound elastase of neutrophil extracellular traps degrades plasminogen, reduces plasmin formation, and decreases fibrinolysis: proof of concept in septic shock plasma. *FASEB J.* **33**, 14270–14280, <https://doi.org/10.1096/fj.201901363RRR>
- 53 Nebulised Dornase Alfa for Treatment of COVID-19 (COVASE). <https://clinicaltrials.gov/ct2/show/NCT04359654>
- 54 Efficacy and Safety of Aerosolized Intra-tracheal Dornase Alpha Administration in Patients With COVID19-induced ARDS (COVIDORNASE). <https://clinicaltrials.gov/ct2/show/NCT04355364>