Arteriosclerosis, Thrombosis, and Vascular Biology

EDITORIAL

Neutrophil Extracellular Traps as Prognostic Markers in COVID-19

A Welcome Piece to the Puzzle

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The article by Ng et al[1] is highlighting the presence of circulating neutrophil extracellular traps (NETs) in coronavirus disease 2019 (COVID-19) and their role as prognostic indicator. The initial description of NETs in plasma of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected patients in April 2020[2] has focused interest in neutrophil function and NET formation in this condition. Severe COVID-19 cases are putting pressure on health care systems and particularly on intensive care units with median lengths of intensive care unit stay ranging from 6 to 12 days in studies conducted in China and 4 to 19 days in studies outside of China.[3] But which features make NETs determinants of clinical outcome and do we actually have sufficient data to support a specific role in COVID-19?

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The concept of NETosis was introduced in 2004[4] as release of extracellular DNA traps by neutrophils, composed of decondensed chromatin and granule proteins. NETosis-inducing agents (Figure) are bacteria, fungi, protozoa, viruses, platelets, cytokines, and nitric oxide donors. NET formation is a form of cell death[47] involving the translocation of elastase and myeloperoxidase from primary granules to the nucleus where they cleave histones after hypercitrullination catalyzed by PAD-4 (peptidylarginine deiminase 4), leading to chromatin decondensation.[48] Although NET generation has been described initially as an antimicrobial mechanism, recent data suggest that NETs contribute to lung injury,[30,49] vascular thrombosis,[50] and multiple other conditions (Figure).

Circulating surrogate markers of NETs in plasma are complexes of DNA and myeloperoxidase,[2,9,17,51–53] citrullinated histone H3,[2,51,53] cell-free DNA,[2,51,53] and neutrophil elastase.[51] The data of Ng[1] are based on a relatively large patient number including 5-month follow-up samples compared with previous studies. However, for the assessment of outcomes, robust statistical methodology will be needed, with multivariate analyses of large sample sets corrected for confounders, such as age and cardiovascular risk factors.

Drastic changes in blood neutrophils can originate from mobilization of neutrophils from the marginated pool of the lung via CXCR4 (C-X-C motif chemokine receptor 4)–CXCL12 (C-X-C motif chemokine ligand 12) interactions leading to a spill-over of the pulmonary inflammatory process to the systemic circulation.[54] Authors' observation that circulating NETs markers correlate with markers of inflammation and endothelial damage in COVID-19[1] emphasize the relevance of the virus for the vasculature, and centers the causes for patients' demise on the microvascular thrombosis aspect of the infection.[50] Although SARS-CoV-2–derived mRNA may not be detectable in blood during active infection,[55] the virus is able to directly infect activated neutrophils via surface ACE-2 (angiotensin-converting enzyme 2).[17] Authors demonstrate derangement of the endothelial activation/damage marker VWF (von Willebrand factor) and its protease, ADAMTS13 (a disintegrin and metalloproteinase

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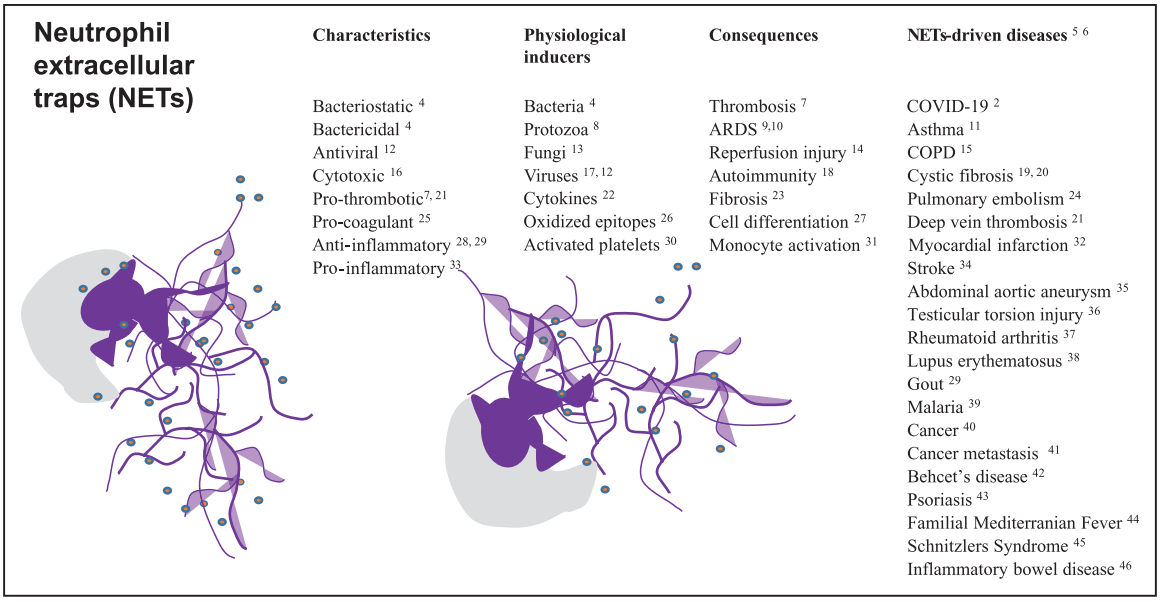

Figure. Neutrophil extracellular traps (NETs) shed from activated neutrophils (neutrophil body in gray, nucleus and NETs in purple, schematic drawing), and NETs-driven diseases.
ARDS indicates acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; and COVID-19, coronavirus disease 2019.

with thrombospondin motifs 13),[1] which is an elegant suggestion of endothelial injury and potential microthrombosis that is in proportion with markers of NETs. Mechanisms on how virus/various-length-pieces of circulating DNA exert ADAMTS-13 suppression are unknown.

The lung retains primed neutrophils, a protective mechanism shown to be impaired in acute respiratory distress syndrome.[56] NETs released by SARS-CoV-2–activated neutrophils promote lung epithelial cell death in vitro[17] and neutrophil infiltration. Interstitial NETs and intravascular thrombi are characteristic features of acute respiratory distress syndrome lungs in lethal COVID-19 cases.[17,57–59] NETs were found in airways together with fibrin occluding alveoli and bronchioles.[57] NETs trigger coagulation[60] and foster fibrin deposition in the airways compromising pulmonary ventilation and gas diffusion capacity. In accordance, Ng et al[1] show that higher circulating NETs levels are associated with the need for respiratory support and with mortality, which confirms smaller studies.[9] By contrast, other reports indicated that ventilator-dependent patients exhibited higher concentrations of cell-free DNA[2,51] which signifies general cell death and is not specific for neutrophils.

Establishing causality between NETs burden and poor outcome highlights an urgent need for representative models of SARS-CoV-2 infection. So far, ferrets and hamsters are reported to come closest to humans, considering virus replication, clinical signs, pneumonia, transmission, immunology, and demographics.[61] However, all available models to date seem to lack formation of NETs and lung thrombosis,[62] suggesting that they do not serve to study severe SARS-CoV-2 infection.

Circulating deoxyribonucleases (DNase) may be another important puzzle piece in COVID-19. Deoxyribonucleases 1 and 1L3 are naturally regulating the amount of circulating extracellular chromatin, and intact endogenous plasma DNase activity is essential for homeostasis and survival.[63] No data on DNase activity in patients have been published, leaving us puzzled about its association with disease severity and potential effects on circulating NETs markers. For the full picture, authors should analyze DNase activity in their samples.

Directly targeting NETs by deoxyribonucleases has been proposed as a therapeutic approach in COVID-19, even before the first data on circulating NETs markers had been published.[59] Eight trials are currently registered on ClinicalTrials.gov to test the effect of NETs degradation, whereby 6 are recruiting patients with respiratory failure/acute respiratory distress syndrome. Design and outcome of these studies will likely impact our view on the role of NETs in SARS-CoV-2 pathophysiology. There are still many hurdles to take. What if there exists a significant component of immune-mediated, virus-independent immunopathology as a primary mechanism in severe disease, do NETs still play a role? Immunosenescence of neutrophils is only partially understood, but inaccurate chemotaxis and reduced pathogen clearance are expected to result in increased tissue damage.[64] These observations could affect treatment success and effectiveness and might require prospective stratification of analyses. In addition, dynamics of degradation and formation of cleavage products are likely to differ between compounds and routes of administration. Inflammatory responses of monocytes to chromatin depend on fragmentation into mononucleosomes and dinucleosomes,

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which are histones still wrapped in DNA.[31] The complexity of synergistic signaling by citrullinated nucleosomes goes beyond the cytotoxicity of circulating naked histones.[31] Authors' observation that elevated circulating NETs markers are prognostic indicators for outcomes in patients with COVID-19[1] is a simple and welcome puzzle piece in a tricky setting.

ARTICLE INFORMATION

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