Neutrophil extracellular traps and thrombogenesis in COVID-19 patients

Ahmed Yaqinuddin

Department of Anatomy and Genetics, College of Medicine, Alfaisal University, Riyadh, Kingdom of Saudi Arabia

COVID-19 has caused significant morbidity and mortality around the world. Recent reports point toward the "cytokine storm" as core of pathogenesis in SAR-CoV-2-induced acute lung injury, acute respiratory distress syndrome (ARDS), coagulopathy, and multiorgan failure. We have presented clinical data here wherein cytokine levels in COVID-19 patients do not match typical cytokine storm seen in ARDS. Interestingly, COVID-19 patients in early disease present with hypoxemia with no significant respiratory dysfunction. In addition, it is reported that hospitalized COVID-19 patients have a high incidence of thrombotic complications, especially involving the pulmonary vasculature. We hypothesized that core to pathogenesis of COVID-19 is the dysregulation of neutrophils, which culminates in excessive release of neutrophil extracellular traps (NETs). Recently, an increasing amount of NETs have been seen in sera of severe COVID-19 patients. We have discussed here mechanisms involved which lead to thrombogenesis and vasculitis because of excessive release of NETs.

Key words: Coronavirus, COVID-19, neutrophil extracellular traps, neutrophils, SARS, thrombosis, vasculitis

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INTRODUCTION

Severe acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) observed following SARs-CoV-2 infection have been attributed to the disruption of the host inflammatory and immune response.^[1] However, patients diagnosed with COVID-19 exhibit early hypoxemia with minimal respiratory dysfunction, alongside reduced lymphocytes with neutrophilia.^[2] Lower CD8⁺ cell levels are also observed such that CD8⁺/neutrophil and neutrophil/lymphocyte ratios are predictive of disease outcome. Increased pro-inflammatory cytokines including IL-6, IL-1 β , and TNF- α have also been noted in moderate and severe COVID-19 cases.^[1] Collectively, such findings suggest neutrophilia with an associated moderate increase in cytokine levels being core to disease pathogenesis.^[1] Indeed, vasculopathy and coagulopathy accompany cytokine elevation, with a high incidence of thrombotic complications observed

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in critically ill COVID-19 patients.^[3] Segmental hyperperfusion with vasodilation and endothelial dysfunction in the pulmonary vasculature of COVID-19 patients has been observed, while further reports indicate increases in pulmonary dead space that is attributable to pulmonary microthrombosis and embolism.^[4] However, moderate increase in cytokine levels does not match expected levels seen in typical ARDS (i.e., a cytokine storm) [Table 1].^[1,5,6] In this manuscript, we evaluate the role of neutrophil extracellular traps (NETs) in causing thrombosis in COVID-19 patients.

METHODS

This article is based on a critical analysis of literature on peer-reviewed article indexing databases including PubMed, Scopus, and Medline, using NETS, thrombosis, ARDS, and COVID-19 as keywords. Data and relevant aspects were compiled and presented as discussion points in the manuscript.

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Address for correspondence: Dr. Ahmed Yaqinuddin, College of Medicine, Alfaisal University, Riyadh, Kingdom of Saudi Arabia. E-mail: ayaqinuddin@alfaisal.edu

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| syndrome and COVID-19 patients | | | | | |
|--------------------------------|--|---------------------------------------|--|---|--|
| Cytokines | Moderate ARDS (<i>n</i> =13) (pg/ml) | Severe ARDS (<i>n</i> =5) (pg/ml) | COVID-19 (nonsevere) (<i>n</i> =166) (pg/ml) | COVID-19 (severe) (<i>n</i> =286) (pg/ml) | |
| IL-1β | 552.5 | 610.9 | 5.0 | 5.0 | |
| IL-6 | 856 | 345 | 13.3 | 25.2 | |
| TNF-α | 46.4 | 41.4 | 8.4 | 8.7 | |
| IL-8 | 189.2 | 187.3 | 13.7 | 18.4 | |

Table 1: Comparison of recorded specific cytokine levels in cases of moderate and severe acute respiratory syndrome and COVID-19 patients

Data modified from Nguyen et al. (2018) and Qin et al. (2020). ARDS=Acute respiratory syndrome; IL=Interleukin; TNF=Tumor necrosis factor

RESULTS AND DISCUSSION

Neutrophil extracellular traps and COVID-19

NETs are web-like structures consisting of DNA, histones, toxic protein granules, and enzymes, which are released from neutrophils as they undergo a specialized type of cell death called "NETosis." These unique structures are now increasingly associated with a central role during COVID-19 pathogenesis.^[7] Although NETs primarily trap microbes and associated debris, uncontrolled NET proliferation culminates in alveolar damage, endothelial injury, and coagulopathy.^[8] NETosis is a well-orchestrated process, whereby neutrophils undergo morphological alterations in response to infections, platelets, and inflammatory mediator, resulting in nuclear membrane rupture.^[8] This results in mixing of nuclear components (DNA and histones) with cytoplasmic granular content including myeloperoxidase and neutrophil elastase (NE).^[8] Although NET release during infections is physiologically beneficial, excessive NET production could prove harmful, resulting in tissue injury and thrombosis.[8] Indeed, NET-specific markers are significantly elevated in COVID-19 patient sera, while such sera triggered NET production in healthy control-derived neutrophils.^[7] It is thus possible that ALI, vasculopathy, and coagulopathy seen in COVID-19 patients could be due to excessive release of NETs from neutrophils.

It is increasingly clear that effective neutrophil apoptosis leads to resolution of the inflammatory response in particularly respiratory conditions and perhaps may represent a more proinflammatory form of apoptosis.^[9] Indeed, NETs in pneumonia do not seem to participate in bacterial clearance but rather contribute to compromised lung function with higher levels of NETs in blood associated with adverse outcomes.^[10] Thus, while specific numbers and statistics connecting NETs and respiratory syndromes are yet to be established, high NET levels have been associated with increased duration of treatment and inpatient stay, a prolonged timeline to recovery, and increased mortality in severe pneumonia.^[9-13] Indeed, persistent low concentrations of circulating NETs were associated with better outcomes.^[11] **Neutrophil extracellular trap overproduction may underlie thrombosis and vasculitis in COVID-19 patients** Intact NETs exert a significant role during coagulation, whereby platelets become entangled within the NET web-like extrusion.^[14] This results in platelet activation and aggregation.^[14] Furthermore, NETs induced thrombin formation by developing scaffolds that trap pro-thrombogenic factors including red blood cells, fibrin, fibronectin, von Willebrand factor, factor XII, tissue factor (TF), and endovesicles containing TF.^[14] Excessive NET release culminates in thrombogenesis both with and without the presence of fibrin, which is evidenced by NETs forming scaffolds with large aggregates, capable of blocking microvasculature without activation of coagulation pathways and thrombus formation.^[14]

Furthermore, individual NET components have also been reported to induce thrombosis. Histone-DNA complexes containing thrombi are more stable than fibrin-containing scaffolds, which significantly prolong clot lysis time and can be shortened using deoxyribonuclease 1, preventing clot formation altogether.^[14] DNA within NETs can directly activate Factor XII, initiating the intrinsic coagulation cascade, while NET histones increased TF expression in endothelial cells and macrophages, thus activating the extrinsic coagulation cascade.^[14,15] Furthermore, histones can activate platelets through Toll-like receptors (TLRs) 2 and 4, culminating in platelet aggregation by fibrinogen recruitment.^[14] Strikingly, activated platelets can stimulate neutrophils to release NETs via TLR4, P-selectin, and high mobility group box 1.^[14]

NET components, such as cathepsin G and NE, may also degrade anticoagulants involved in the inhibition of TF. Histones bind to thrombomodulin, inhibit the activity of protein C, and thus inhibit the activation of protein C.^[14] Finally, DNA in NETs interferes with degradation of fibrin by plasmin, due to its tight interactions with them.^[14] Most importantly, intact NETs have been identified in both arterial and venous thrombi, forming an integral part of thrombi in histological examination of tissues derived from patients with myocardial infarctions, strokes, and pulmonary embolism.^[14] Dysregulation and excessive NET production can also promote the production of antineutrophil cytoplasmic antibodies (ANCAs) and

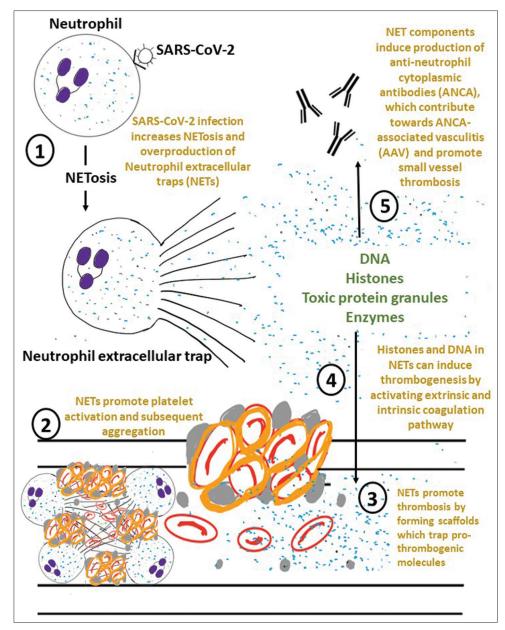


Figure 1: Schematic visualization of the proposed mechanism underlying increased neutrophil extracellular trap production in response to SARS-CoV-2 infection, leading to thrombosis and antineutrophil cytoplasmic antibody-associated vasculitis (steps 1–5). Viral infection elevates the production of neutrophil extracellular traps and their constituents. Neutrophil extracellular traps form stable scaffolds that trap platelets, red blood cells, fibrinogen, and fibronectin, preventing their degradation and promoting coagulation, leading to thrombosis. Concurrently, individual neutrophil extracellular trap components induce thrombosis by activating intrinsic and extrinsic coagulation pathway. Finally, neutrophil extracellular trap components give rise to numerous antineutrophil cytoplasmic antibodies that will lead to antineutrophil cytoplasmic antibody-associated vasculitis, particularly in small vessels, and promote thrombosis in small vessels

cause vasculitis.^[16] These groups of antibodies result in ANCA-associated vasculitis, which affects small vessels, and it is accompanied by elevated ANCA levels in patient serum.^[16]

Although most COVID-19 patients exhibit mild flu-like symptoms, 5%–10% of cases involve life-threatening pneumonia and respiratory failure. Studies of patients with SAR-CoV infections implicated the involvement of cytokines IL-1 β , TNF- α , and IL-6 as causative factors.^[17] IL-1 β induces the generation of other cytokines, including

IL-6 and TNF- α , thereby contributing to the "cytokine storm" of inflammatory diseases.^[18,19]

CONCLUSION

We suggest that excessive NET release from neutrophils centrally underlies SARS-CoV-2 infection and COVID-19 pathogenesis. Excessive NET release has been associated with alveolar damage and accumulation of edema, endothelial injury and coagulopathy, elevated platelet activation, and thrombogenesis [Figure 1].^[8] Considering our hypothesized mechanism of pathogenesis of COVID-19 which remains as yet poorly understood, sera and bronchoalveolar lavage of COVID-19 patients need to be evaluated for levels of specific biomarkers for NETs.^[7,20] Furthermore, therapeutic strategies should be developed based on controlling/reducing excessive NET production and countering the potential effects of such elevations.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- 1. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, *et al*. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis 2020;71:762-8.
- Gattinoni L, Coppola S, Cressoni M, Busana M, Rossi S, Chiumello D. COVID-19 Does not lead to a "Typical" acute respiratory distress syndrome. Am J Respir Crit Care Med 2020;201:1299-300.
- Klok FA, Kruip MJ, van der Meer NJ, Arbous MS, Gommers D, Kant KM, *et al.* Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: An updated analysis. Thromb Res 2020;191:148-50.
- Caruso D, Zerunian M, Polici M, Pucciarelli F, Polidori T, Rucci C, et al. Chest CT features of COVID-19 in Rome, Italy. Radiology 2020;296:E79-85.
- Nguyen LN, Tran DH, Dong KH. Prognosis value of serum cytokine levels among burn-induced ards patients. Ann Burns Fire Disasters 2018;31:185-8.
- 6. Owlia S, Owlia MB. Glucocorticoids in COVID19; a friend not foe. J Res Med Sci 2020;25:45.
- Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, et al. Neutrophil extracellular traps in COVID-19. JCI Insight 2020;5(11): e138999.
- Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, *et al*. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med 2020;217(6): e20200652.

- Twaddell SH, Baines KJ, Grainge C, Gibson PG. The emerging role of neutrophil extracellular traps in respiratory disease. Chest 2019;156:774-82.
- Ebrahimi F, Giaglis S, Hahn S, Blum CA, Baumgartner C, Kutz A, *et al*. Markers of neutrophil extracellular traps predict adverse outcome in community-Acquired pneumonia: Secondary analysis of a randomised controlled trial. Eur Respir J 2018;51:1701389.
- Bendib I, de Chaisemartin L, Mekontso Dessap A, Chollet-Martin S, de Prost N. Understanding the role of neutrophil extracellular traps in patients with severe pneumonia and ARDS. Chest 2019;156:1278-80.
- 12. Mikacenic C, Moore R, Dmyterko V, West TE, Altemeier WA, Liles WC, *et al.* Neutrophil Extracellular Traps (NETs) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. Crit Care 2018;22:358.
- Lefrançais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. JCI Insight 2018;3:e98178.
- 14. Thålin C, Hisada Y, Lundström S, Mackman N, Wallén H. Neutrophil extracellular traps: Villains and targets in arterial, venous, and cancer-associated thrombosis. Arterioscler Thromb Vasc Biol 2019;39:1724-38.
- Vu TT, Leslie BA, Stafford AR, Zhou J, Fredenburgh JC, Weitz JI. Histidine-rich glycoprotein binds DNA and RNA and attenuates their capacity to activate the intrinsic coagulation pathway. Thromb Haemost 2016;115:89-98.
- 16. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. Nat Rev Rheumatol 2019;15:91-101.
- 17. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, *et al.* Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 2020;9:221-36.
- 18. Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, *et al*. COVID-19 infection: The perspectives on immune responses. Cell Death Differ 2020;27:1451-4.
- Yaqinuddin A, Kashir J. Novel therapeutic targets for SARS-CoV-2-induced acute lung injury: Targeting a potential IL-1β/neutrophil extracellular traps feedback loop. Med Hypotheses 2020;143:109906.
- 20. Cicco S, Cicco G, Racanelli V, Vacca A. Neutrophil Extracellular Traps (NETs) and Damage-Associated Molecular Patterns (DAMPs): Two potential targets for COVID-19 treatment. Mediators Inflamm 2020;2020:7527953.