**Netosis -A double-edged sword in the Pathogenesis of LONG COVID**

**Abstract:**

The emergence of COVID-19 as a global pandemic has had far-reaching effects on the health of individuals worldwide. Although there has been a decrease in the severity of the disease, there is a growing concern about the long-term impact of COVID-19 on the health of individuals, particularly cardiovascular complications, known as Long-COVID, which can significantly increase morbidity and mortality rates in people recovering from COVID-19 in the recent past.

The severity of COVID-19 has been linked to various factors, including the role of neutrophils and neutrophil extracellular traps (NET). These extracellular webs, composed of chromatin, microbicidal proteins, and oxidant enzymes, are released by neutrophils to fight infections. However, if not properly regulated, NETs can lead to thrombo-inflammatory states and microangiopathy in the body, resulting in complications such as sepsis, thrombosis, and respiratory failure.

Understanding the detailed pathophysiology and association of NETs with the prognosis of COVID-19 infection is crucial for future implications and management.

The purpose of this review is to analyze the potential contribution of NETosis in the pathophysiology of COVID-19 and its subsequent complications apart from its beneficial effect. This may provide insight into potential therapeutic interventions for COVID-19 patients.

**Keywords:**  COVID-19, heart, long-COVID, post-acute sequelae of COVID-19, SARS-COV-2, Innate immunity, NETS, Netosis.

**Introduction**

In December 2019, SARS-CoV-2 emerged and quickly spread worldwide, with its high infectivity and numerous ongoing mutations, including the latest variant XBB.1.5 1. As on 6 April 2023, there are 762,201,169 confirmed cases of COVID-19, including 6,893,190 deaths, reported to WHO.2 The number of COVID-19 cases that remain asymptomatic in many ranges anywhere from 18-80%, while up to 15% can go on to develop severe disease.3

While the severity of COVID-19 appears to be decreasing globally, there has been a recent trend of increased incidence of cardiac morbidity and mortality. This includes an elevated risk of various cardiovascular problems, such as heart muscle inflammation, blood clots, abnormal heart rhythm, strokes, myocardial infarction, and heart failure, among individuals who have recovered from COVID-19. 4

Notably, this increased risk is observed even in individuals who were previously healthy, non-obese, and non-smokers and had no preexisting conditions such as kidney disease or diabetes. 5

Patients who have recovered from severe forms of COVID-19 often experience a lengthy and challenging recovery process after spending time in the intensive care unit. This recovery period can be marked by persistent systemic inflammatory symptoms and residual effects. COVID-19 survivors commonly report symptoms such as headaches, fatigue, joint pain, and tissue damage to organs such as the lungs, heart, and skin.4 The complications may result from various factors, including thrombosis and disseminated intravascular coagulation, as well as crosstalk between hyperactivated neutrophils and complement system proteins and platelets.

In addition, COVID-19 can also cause disorientation in the adaptive immune response and lead to a cytokine storm that weakens the immune system, potentially exacerbating the severity of the infection.

There is mounting evidence to suggest that neutrophils may play a key role in driving many of these processes, including the hyperactivation of the immune system and the development of various COVID-19-associated complications.6 The severity of COVID-19 has been investigated to be linked to a variety of factors, of present interest being the role of neutrophils and neutrophil extracellular traps (NET).5

Some studies have found a high neutrophil-to-lymphocyte ratio in patients with critical disease and have found it to predict patient morbidity and mortality.7,8,9 Other studies have found the lungs of deceased patients with COVID-19 to be infiltrated with neutrophils, both in the air spaces as well in the pulmonary capillaries.10 Unregulated reactive oxygen species (ROS) production by neutrophils can lead to RBC membrane peroxidation, thrombosis and alveolar damage.11

Neutrophils play a key role in defence against pathogens - they achieve this in ways; first by the oxidative burst taking place within the phagolysosome of the cell and the other being the release of NETs to contain the infection.6 Both these processes if not properly regulated function as a double-edged sword.

NET is considered an important factor which impacts the pathophysiology and clinical presentation of COVID-19 and LONG COVID. In the absence of a specific and effective treatment, it is crucial to understand the interplay of factors including the crucial role of NETs in the body that brings about the disease findings.6

NETosis refers to a unique type of active cell death in neutrophils, which is marked by specific morphological features like loss of plasma membrane, indistinct nuclear envelope, and decondensed chromatin.NETs are extracellular webs of chromatin, microbicidal proteins and oxidant enzymes that are released by neutrophils to combat infection; but if these aren’t properly regulated, have been proven to be a cause of a thrombo-inflammatory state.12

It is now well known that COVID-19 sera are found to contain abundant NETosis markers such as cell-free DNA, myeloperoxidase- (MPO-) associated DNA, and citrullinated histone H3 (citH3).13

It is becoming increasingly evident that post-acute COVID-19 syndrome is a distinct entity characterized by persistent and often severe complications, which may last for an extended period. This highlights the need for its recognition as a clinical condition.14

Another important prognosticator of severe COVID-19 patients is abnormal coagulation. Several studies have investigated and found this to be related to the decrease in platelet number, increased fibrin degradation products, increased neutrophil counts and increased neutrophils to lymphocyte ratio.15,16  NETs have been found to play a role in enhancing inflammation and microangiopathy in severe COVID-19 cases.17

Severe cases of COVID-19 infection are associated with unregulated NET formation occurring across multiple sites leading to blood vessel narrowing also seen in the lungs with ARDS.18 NETs cause further activation of neutrophil-stimulating cytokine IL-3 from macrophages, creating a vicious loop of further NET formation.19 Previous research has also investigated the role of NETs in the activation of T cells thus further enhancing inflammation. 20 Several post-mortem studies have highlighted platelet and fibrin-rich thrombus which also contain neutrophils, neutrophil extracellular traps (NETs) and activated factor XII (FXII) that triggers the contact pathway, throughout pulmonary vasculature.21,22

In the past, various studies have concluded that cytokine storm associated with acute respiratory distress syndrome is in a significant way associated with the excess of neutrophil extracellular traps (NETs) formation.21 There is also prominent endothelial dysfunction and thrombosis in this condition. A growing number of studies have added to this assumption, also concerning potential treatment options.

These observations raise concerns about the limits of our current knowledge and understanding of the potential cause of COVID-19 severity and its long-term complication This review was performed to analyze the role of NETosis in the pathophysiology of COVID-19 and its sequelae, famously known as Long COVID.

**SARS-CoV 2**  and **Pathology of COVID-19**

SARS-CoV-2 is a member of the family of coronaviruses;Coronaviridae. order: nidovirales genus: beta coronavirus and lineage B of subgenus: sarbecovirus23,24  It is a type of positive sense RNA virus with helical nucleocapsid and enveloped virion. Of the seven coronaviruses known to cause respiratory disease in humans, four rarely cross the species barrier and are endemic to humans causing the common cold. While the other three are zoonotic viruses whose primary hosts are bats, these have crossed the species barrier to cause deadly diseases in humans on the pandemic level. SARS-CoV-1 was identified in 2002 causing the severe acute respiratory syndrome, In 2012 came MERS-CoV which causes Middle East Respiratory Syndrome, and lastly in 2019 SARS-CoV-2 which causes coronavirus disease-19 or COVID-19 .25 SARS-CoV-2 was so named because it shares a genetic sequence with SARS-CoV-1 with a whopping 79% overlap.26 Variants of concerns since the start of the pandemic included Alpha, Beta, Gamma and Delta (SARS-CoV-2 lineages/ Pango Lineages of concern: B.1.1.7, B.1.351, P.1, and B.1.617.2 respectively). Currently, the predominant “Variant of concern” circulating the world over is Omicron variant XBB.1.5.1,27,28

“Corona” in Latin means the crown which is because of the crown-like spikes of S protein on its surface.  In SARS-CoV-1 and SARS-CoV-2 the protruding portion of the S protein is heavily glycosylated and is involved in receptor binding, membrane fusion and entry into host cells. The latter has a higher receptor affinity than the former.29-32The Spike protein binds to ACE-2 receptors which are most numerous in type-2 alveolar cells in the lungs, also present on small intestinal epithelial cells, vascular endothelial cells, arterial smooth muscle cells, immune cells and neurons explaining the multiorgan manifestations of the disease.33,34ACE-1 is a homolog of ACE-2 and both these serve essential functions in the renin-angiotensin-aldosterone axis. ACE 1 cleaves Ang 1 to Ang 2 which is involved in the pathogenesis of hypertension and atherosclerosis. Whereas, ACE 2 cleaves Ang 2 into Ang 1-7 which serve vasodilatory, anti-inflammatory and anti-atherosclerosis functions through Mas receptor binding.35-38 The relative expression of ACE 1 and ACE 2 thus determines the relative abundance of Ang 2 and Ang 1-7. **(Table 1,2)**

**COVID -19 and its sequelae (Post acute sequelae of COVID-19)**

Following recovery from severe forms of COVID-19, patients often experience a prolonged and difficult convalescence period lasting several days or even weeks. During this time, they may continue to experience residual systemic inflammatory symptoms and other sequelae of the disease. Common symptoms experienced by COVID-19 survivors during this phase include headaches, weakness, muscle pain, and tissue damage to the lungs, heart, and skin.

This process is found not just in older patients but also in young ones. People having diabetes, chronic obstructive pulmonary disease (COPD), cardiovascular diseases, hypertension, other malignancies, and immunocompromised have more propensity to develop a serious infection.4 (Table 1,2)

In addition, patients may also experience neuro psychopathologies, such as cognitive impairment and mood disorders. Interestingly, COVID-19 shares many features with other viral immune-driven diseases, including hyperinflammatory immune responses, coagulation disorders, and painful convalescence.4 Infections like the Ebola virus, Dengue fever, and West Nile fever have been associated with post-infection residual inflammation and autoimmune-like relapses, resulting in conditions such as rheumatoid arthritis, arthralgia, spondyloarthritis, and uveitis. 49

Research conducted by Wilson HW has shown that Post-Ebola Syndrome can affect up to 90% of survivors, particularly women (67%), within the first 1-12 weeks of being discharged from hospitalization. 49

The detection of autoantibodies in the blood of survivors, including anti-nuclear antibodies, anti-CCP, and rheumatoid factor, strongly suggests that autoreactivity is still involved in the persistent inflammation and tissue damage that occurs during the recovery period. 50 similar observations have been reported in cases of Dengue fever and West Nile fever, albeit to a lesser extent. 50,51These findings postulate that there could be similar autoimmune complications in COVID-19 and female population could be suffering more .4

**COVID-19 and the immune system**

The body has its immune system to tackle various infections and pathogens. The main defence system consists of the white blood cells, lymph node, spleen and draining lymphatics which contain specialized compartments to encounter antigens. When the body encounters any pathogen for the first time, the immune system cannot identify the pathogen which can lead to infection getting converted into disease. This occurred with COVID-19 infections as there was no memory of the infection with our immune system.52 With no proper cure treatment available the immune system remains the best defence against COVID-19. Once the virus enters the body, the immune surveillance responds to form antibodies. The B cells get assisted by T cells to differentiate into plasma cells, which produce antibodies specific to block the virus from entering into host cells, curtail the infection and prevent further recurrence of infection. Although, the data on SARS-CoV-2 remain limited. There are many possible mechanisms through which SARS-CoV-2 evades the immune system.53 Inhibition of type 1 interferon in early disease along with a suppression of the innate response. Compared with SARS-CoV and other closely related coronaviruses, its S protein is 20–30 amino acids longer.SARS-CoV-2 has similar immune evasion strategies, along with it also has an additional mechanism which remains undiscovered.54

Elevated numbers of monocytes, neutrophils and neutrophil-to-lymphocyte ratio are sen in impaired blood vessels and are associated with a bad prognosis of the disease.55 NETosis is a complex immune response that involves the activation of neutrophils to expel nuclear and cytoplasmic proteins into tissues and the blood. While this process can be protective in terms of lysing pathogens, it can also lead to tissue damage and the release of auto-antigens and danger-associated molecular patterns (DAMPs).

The release of these molecules can induce inflammation and immune stimulation, which can further perpetuate tissue damage. However, efficient clearance of these self-antigens through efferocytosis can help maintain tissue tolerance and limit inflammation-related tissue damage. When NETosis is excessive and not efficiently cleared by efferocytosis, it can lead to infection-induced systemic inflammatory response syndrome (SIRS). During SIRS, immune cells are activated to eradicate pathogens, and this bystander activation can also facilitate the activation of autoreactive cells, potentially leading to autoimmune disease. However, resolution of the SIRS, cleaning of tissue damage and NETs, and finally suppressive brake by inducible regulatory cells can prevent long-lasting autoimmune disease.56

**Neutrophils and Neutrophil extracellular traps**

Neutrophils are the most abundant white blood cell fraction in blood. Neutrophils along with Neutrophil extracellular traps are a key component of the innate immune system and serve as the first line of defence against infection. Before 2004, phagocytosis was considered the main mechanism by which neutrophils attack invading pathogens, however, in 2004 Brinkman et al discovered neutrophil extracellular traps which serve as an alternative to phagocytosis in the eradication of pathogens.58 Neutrophil extracellular traps or NETosis is a distinct form of active cell death in neutrophils, characterized by morphological changes such as loss of plasma membrane, decondensed chromatin, and indistinct nuclear envelope. This phenomenon is termed NETosis, and it differs from other forms of cell death such as necrosis and apoptosis. While initially considered a beneficial host defence mechanism, recent studies have revealed harmful aspects of NETosis. Excessive NET formation resulting from NETosis can damage vascular endothelial cells, impair diabetic wound healing, and form thrombi.

Additionally, NET dysregulation has been linked to autoimmune diseases such as systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody-associated vasculitis, and psoriasis, while they can promote tumour growth and interfere with cytotoxic lymphocytes, they also play a positive role in innate immunity. These diverse functions have made NETs a topic of great interest for researchers.6

A major enzyme involved in neutrophil extracellular trap formation is peptidyl arginine deiminase 4(PAD4), which causes the citrullination of histones molecules..59However PAD4 independent and reactive oxygen species(ROS) stimulated pathways of NETs formation have been discovered in vitro studies.60

Cit-H3, MPO DNA and cell-free DNA are common markers of NETosis. Cit-H3 levels in one of the studies did not correlate well with the other two markers, but rather were strongly associated with platelet count– this isn’t surprising considering the pathway leading to Cit-H3 formation is linked to platelets at some point.61

Inflammatory states such as infection can cause neutrophilia as well as changes in neutrophil morphology such as left shift, toxic granulations, cytoplasmic vacuoles, etc. Research has shown an increased number of neutrophils during COVID-19 infection.62,63 An increase in the number of immature neutrophils was also reported, as seen in bacterial sepsis. Some studies have shown this increase in neutrophils along with an increase in the neutrophil chemo-attractant IL-8 in COVID-19 patients to be associated with poor prognosis.19,64-65, An increase in circulating NETs was also seen in COVID-19 patients of any severity.45,47

NETopathies such as COVID-19 can cause an accumulation of neutrophils in the lung vasculature, as well as increased levels of interferon, lactate dehydrogenase (LDH), C-reactive protein (CRP), pro-inflammatory cytokines, and circulating fibrinogen. These factors can lead to respiratory failure, potentially progressing to acute respiratory distress syndrome (ARDS), sepsis, thrombosis, acute cardiac injury, and even heart failure.42

Circulating by-products of neutrophil extracellular traps (NETs), such as circulating nuclear DNA (cir-nDNA), circulating mitochondrial DNA (cir-mtDNA), histones, and granules like neutrophil elastase (NE) and myeloperoxidase (MPO), can trigger the inflammatory process. This can result in complex diseases characterized by cytotoxicity towards endothelial and epithelial cells, prothrombotic activity, and abnormalities of coagulation factors leading to systemic vascular permeability.66

Exaggerated and uncontrolled formation of NETs may result in MI, vasculitis, haemorrhage and multiple organ malfunction. In the case of NETopathies such as COVID-19, there is an excessive concentration of neutrophils in the lung vasculature, as well as elevated levels of interferon, lactate dehydrogenase (LDH), C-reactive protein (CRP), pro-inflammatory cytokines, and circulating fibrinogen. These factors can induce respiratory failure up to the point of acute respiratory distress syndrome (ARDS), as well as sepsis, thrombosis, acute cardiac injury, and even heart failure.66

Invitro studies have shown a higher baseline NET production by neutrophils of COVID-19-infected patients. There was a greater abundance of NETs in tracheal aspirate when compared to plasma.68 Neutrophils have also been found to infiltrate the lungs of hospitalized COVID-19 patients.46,6 Interestingly, one of the studies reported NETs to infiltrate only the interstitial spaces and the airways while neutrophils predominated in the pulmonary microcirculation of COVID-19 patients.47

Several studies have found an association between COVID-19 and increased plasma levels of myeloperoxidase (MPO) and neutrophil elastase (NE), as well as elevated amounts of fragmented circulating DNA (cirDNA). A recent study confirmed that neutrophil extracellular traps (NETs) are a significant contributor to the cirDNA of nuclear origin in the circulation and are associated with an increased risk of thrombosis .56

Cell-free DNA, MPO-DNA complexes and citrullinated histone H3 are important markers of Neutrophil extracellular traps in the blood. Cell-free DNA, LDH, and Cit-H3 are however less specific markers of NETosis since they are also released during neutrophil cell death independent of NETosis.70,71 Cell-free DNA and MPO-DNA were found to be increased in COVID-19 patient sera. A statistically significant positive correlation was found between cell-free DNA levels and MPO-DNA levels with the absolute neutrophil count in one of the studies. Whereas, Cit-H3 levels showed a significant positive correlation with platelet counts.16(Table 1,2)

**Immunothrombosis in COVID-19**

Several studies have shown an increased frequency of thrombotic complications in critically ill COVID-19 patients.72-77(Table 1,2)Laboratory findings in COVID-19 infected patients suggest a hypercoagulable state, which includes an increase in D-dimer, increase in fibrinogen, decrease in platelets and an increased prothrombin time.78 Nicholai et al proposed neutrophils, immunogenic platelets and dysregulated coagulation cascade to have a hand in promoting immune thrombosis in COVID-19 patients. This was confirmed when these microthrombi were examined and were found to be composed of platelets, fibrin and major constituents of neutrophil extracellular traps. 3,79 The presence of circulating platelet neutrophil aggregates further substantiates their role in mediating immune thrombosis in COVID-19-infected patients. Blood vessels of deceased patients showed small vessel clots composed of Cit-H3+ MPO+ cells and NETs .80It has been recently found that Eosinophils also have a role in homeostasis and various diseases, including allergy, infection and also possibly COVID-19. Recent research has highlighted the active cytolytic cell death of eosinophils, which leads to the release of eosinophil extracellular traps (EETs) and total cellular contents, known as eosinophil extracellular trap cell death (EETosis). The pathological contribution of EETosis has been further emphasized by the close association of Charcot-Leyden crystals with eosinophilic inflammation. Although there are currently no gold standard methods to identify EETosis, a common feature appears to be the active lysis of eosinophils that releases cell-free granules and a net-like chromatin structure. EETs/EETosis can be visualized using various techniques, including chemical or fluorescence staining, immunostaining, and electron microscopy. However, it is important to note that the visualization of EETs can be greatly influenced by sample preparation, including the extracellular space of EETotic cells and shear flow.81

Another study assessing COVID-19 patients with STEMI showed increased NETs in 100% of the patients. These results when compared with a 2015 study on STEMI showed an increase in NETs in 68% of the patients.39Also compared to the 2015 study their thrombi were composed of fibrin, NETs and polymorphonuclear cell infiltrates rather than of majorly atherosclerotic plaque fragments as seen in plaques of STEMI patients without COVID-19 .39

**NETs and coagulation**

Previous research has shown Neutrophil extracellular traps to play a role in the etiopathogenesis of many COVID-19 complications such as sepsisand thrombosis and respiratory failure.81-87 Zuo et al found neutrophil activation and NETs biomarkers to be profoundly elevated in COVID-19 patients who developed thrombosis compared to those that did not.49 Neutrophil extracellular traps carry oxidant enzymes and cytotoxic histones outside the cells which when present intravascularly can damage vessel wall driving a cascade of thrombosis in arteries, veins and microvessels.88-90These thrombotic events can lead to catastrophic complications in the body’s vital organs like the lungs, heart and kidney.91-92

NETs can lead to thrombus formation both via direct activation of the extrinsic pathway of coagulation as well as by activating the intrinsic coagulation pathway through tissue factor presentation.93-95 Increased NETs in COVID-19 patients have been shown to cause pulmonary and systemic microvessel occlusion.96,97Experiments conducted on phorbol-12-myristate-13-acetate(PMA) treated neutrophils, and further confirmed by DNase and RNase addition, show that neutrophil extracellular traps can enhance thrombin generation both in a platelet-dependent (polyphosphate dependent factor Xll activation) way as well through the intrinsic pathway of coagulation.93Moreover, serine proteases in NETs destroy antithrombin and tissue factor pathway inhibitors which serve as normal brakes in coagulation thus further enhancing a procoagulant state.98

**Cytokine response in COVID-19 associated with NETosis**

Adaptive immune activity is needed for SARS-CoV-2 virus clearance, the innate immunity, like macrophages, in some cases may lead to the severity. Macrophages lead to the production of IL-6, which may contribute to the excessive inflammation in COVID-19 disease. Macrophage Activation Syndrome could lead to high serum levels of CRP, which are not seen in other viral infections. SARS-CoV 2 restrains antigen presentation by downregulating MHC class I and II molecules and, thus, inhibits the T cell-mediated immune responses.99Humoral immune responses also play a substantial role. IgA, IgM and IgG antibodies show similar dynamics in COVID-19 disease. Structural proteins like M protein could be involved in immunomodulation.100It inhibits type I Interferon production by impeding the formation of TRAF3, TANK, and TBK1/IKK complex, as seen in SARS-CoV-2.101The SARS-CoV M protein also showed a unique proteinaceous PAMP-promoting type I interferon response *via* a TLR-related nonclassical TRAF3- independent mechanism.102

sHLH is like a cytokine surge and is proportionate with COVID-19 disease severity. There is increased interleukin (IL)-2, IL-7, interferon-inducible protein 10, granulocyte-colony stimulating factor, macrophage inflammatory protein 1-, monocyte chemoattractant protein 1, and tumour necrosis factor- (TNF-). 103,104A study in Wuhan, China observed elevated ferritin and IL-6, suggesting that mortality could be associated with hyperinflammation.105(Table 1,2)

**Disease severity and treatment**

Due to the absence of any specific effective therapeutic option for the treatment of COVID-19, delving into a better understanding of the pathophysiology of COVID-19 pathogenesis and complications promises considerable hope for improvement. 16,106

NETs have been shown to play a role in enhancing inflammation in autoimmune diseases such as psoriasis, rheumatoid arthritis, and systemic lupus erythematosus. They could also be associated with LONG-COVID or the sequelae of COVID-19. 107

The non-specific effects of the enzymatic proteins released by NETs can lead to an uncontrolled inflammatory response, resulting in tissue pathology. This can include direct cell damage, recruitment of other pro-inflammatory cells and proteins, and the formation of immune complexes that can induce autoantibody production, ultimately leading to tissue damage. Inhibition of NETs has been shown to decrease the severity of diseases and improve overall survival, making it an important target for therapeutic intervention.107 The detection of neutrophil extracellular traps (NETs) could serve as a prognostic tool for patients with COVID-19. However, to use NETs as a screening tool, it is necessary to standardize and define normal and abnormal levels.107

One possible approach to measure NETs is to examine the levels of NET-associated products in the blood such as cfDNA, citH3, NE, and MPO. CitH3 is particularly specific to NETosis and may be a valuable tool for understanding variations in NET levels.57

NETs were found to be increased in COVID-19 patients. 47,106 Also, the levels of NET markers cell-free DNA and MPO-DNA were higher in patients requiring mechanical ventilation than those in room air. Furthermore, NETs markers increased as the oxygenation of the patient dropped by.47,106

However, absolute neutrophil count, unlike the level of NETs, did not show a significant increase in mechanically ventilated patients .103 NETs are found to worsen critical cases of diseases by their tissue-damaging effects.57 Zuo et al found an increased risk of thrombotic events despite prophylactic anticoagulation in COVID-19 patients showing neutrophil and NETs remnants in their sera.47 Inhibiting NETs may decrease the severity of many diseases, improving overall survival.107

Regular measurement of multiple autoantibodies such as anti-histone, ANCA, anti-cyclic citrullinated peptide, rheumatoid factor, and ANETA, are particularly useful for detecting autoimmune disorders. Anti-nuclear antibodies, in particular, can provide valuable information for the diagnosis and management of Post COVID syndrome. 4

Pisareva et al performed a prospective study on 279 Post-COVID patients and compared their results with those of healthy individuals. to investigate the plasma levels of neutrophil elastase, MPO, and circulating DNA of nuclear and mitochondrial origins in mild, severe, and post-acute phase COVID-19 patients. They found that the NETs markers were statistically different in the patient subgroups as compared to healthy individuals, and had high diagnostic power about disease severity.

The diagnostic power of NE, MPO, and cir-nDNA was measured using the Area Under Receiver Operating Curves (AUROC) and was found to be 0.95, 0.97, and 0.64; 0.99, 1.0, and 0.82; and 0.94, 1.0, and 0.93 in nonsevere, severe, and post-acute patient subgroups, respectively. Furthermore, a significant fraction of patients exhibited aCL IgM/IgG and anti-B2GP IgM/IgG positivity, indicating sustained innate immune response imbalance and a prolonged low-level pro-thrombotic potential activity.They concluded that there is a need for monitoring of these markers in all Long COVID-19 individuals following intensive care.108

Further studies are required to establish the normal range of NET-associated products in healthy individuals and to determine how their levels change in response to COVID-19 infection. Once these measurements are standardized, they may be used as screening tools for the early detection of COVID-19 and for monitoring the progression of the disease in infected individuals. Further studies and clinical trials are needed to develop effective strategies to prevent or destroy NETs to nullify their negative effects.

**Conclusion**

Neutrophils are capable of releasing neutrophil extracellular traps in response to various stimuli. These traps, which include intracellular granule components, function by capturing and destroying a wide range of pathogens including the COVID-19 virus. While NETs can have positive effects in terms of controlling pathogens, they also have pro-inflammatory effects that can contribute to a variety of diseases. The components of NETs are nonspecific and can cause tissue injury, either directly or by increasing the pro-inflammatory response. NETs have been implicated in the enhancement of inflammation seen in autoimmune diseases and may also be associated with LONG-COVID or the sequelae of COVID-19. Inhibiting NETs may decrease the severity of many diseases, improving overall survival.

It is crucial to have a comprehensive understanding of NETs, their pathophysiology, and their association with the prognosis of COVID-19 infection for future implications and management. This knowledge will aid in developing effective therapeutic strategies that target NETs and limit their harmful effects, potentially improving patient outcomes and reducing the severity of COVID-19. Therefore, further research is needed to better understand the role of NETs in COVID-19 and to explore novel therapeutic approaches that can mitigate their impact.

**Declarations**

**Ethical Approval**  -not applicable

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**Authors' contributions**

**Dr Vagisha Sharma: Concept,** preparation of the manuscript, and reference search

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**Abbreviations-**

SARS-CoV 2-Severe acute respiratory syndrome virus-2

EETs -Eosinophil extracellular traps

EETosis - Eosinophil extracellular trap cell death.

NETs -Neutrophil extracellular traps NETs

ARDS -Acute respiratory distress syndrome

MPO-Myeloperoxidase

NET -Neutrophil extracellular trap

NETosis - Neutrophil extracellular trap

PAD4-peptidyl arginine deiminase 4

DAMPs -Danger-associated molecular patterns

ROS -reactive oxygen species

SIRS -inflammatory response syndrome

PRR -pattern recognition receptor

TLR -Toll-like receptors

CBC -Complete blood cell

IL-Interleukin

TNF: Tumor necrosis factor

TLC: Total leukocyte count

SARS‑CoV‑2: Severe acute respiratory syndrome coronavirus 2

GGT: Gamma‑glutamyl transpepatientidase

COVID‑19: Coronavirus disease 2019

USG: Ultrasonography

CT: Computed tomography

IHC: Immunohistochemistry

N/A: Not available

ARDS: Acute respiratory distress syndrome

Hb: Hemoglobin

HbA1c: Glycated Hb

WBC: White blood cell

RBC: Red blood cell

ALT: Alanine aminotransferase

CRP: C‑reactive protein

LDH: Lactate dehydrogenase

RV: Right ventricle

LV: Left ventricle hypertrophy

RA: Right atrium

BMI: Body mass index

CAD: Coronary artery disease

MI: Myocardial infarction

ESR: Erythrocyte sedimentation rate

MF: Myocardial ischemia

MH: Myocardial hypertrophy

PMI: Postmortem interval

McH: Myocyte hypertrophy

IF: Interstitial fibrosis

RT‑PCR: Real‑time reverse transcription–polymerase chain reaction

AST: Aspartate aminotransferase

GFR: Glomerular filtration rate

FFPE: Formalin fixed paraffin embedded

EM: Electron Microscopy

PM: post mortem

BNP: Brain natriuretic peptide

INR: international normalised ratio,

NTproBNP: N-terminal pro-B type natriuretic peptide

CK: creatine kinase

IHD: ischaemic heart disease

PCT: procalcitonin

DAD: diffuse alveolar damage

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