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NET-Mediated Pathogenesis of COVID-19: The Role of NETs in Hepatic Manifestations

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Abstract

Some coronavirus disease-2019 (COVID-19) patients exhibit multi-organ failure, which often includes the liver. Indeed, liver disease appears to be an emerging feature of COVID-19 infections. However, the exact mechanism behind this remains unknown. Neutrophil extracellular traps (NETs) have increasingly been attributed as major contributors to various liver pathologies, including sepsis, ischemic-reperfusion (I/R) injury, and portal hypertension in the setting of chronic liver disease. Although vital in normal immunity, excessive NET formation can drive inflammation, particularly of the endothelium. Collectively, we propose that NETs observed to be elevated in severe COVID-19 infection play principal roles in liver injury in addition to acute lung injury. Herein, we discuss the potential mechanisms underlying COVID-induced liver injury including cytopathic effects from direct liver infection, systemic inflammatory response syndrome, and hypoxic injury, encompassing I/R injury and coagulopathy. Further research is required to further elucidate the role of NETs in COVID. This holds potential therapeutic significance, as inhibition of NETosis could alleviate the symptoms of acute respiratory distress syndrome and liver injury, as well as other organs.

Keywords
- COVID-19
- SARS-CoV2
- NETs
- liver disease
- ACE2 receptor
- multi-organ failure

Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-mediated coronavirus disease-2019 (COVID-19) has severely decimated the global health community, with data suggesting a weakened immune system in individuals presenting with increased severity of disease.[1–4] Although asymptomatic in most cases, some critically ill COVID-19 patients exhibit rapid deterioration characterized by life-threatening acute respiratory distress syndrome, neutrophilia, and a cytokine storm resulting in systemic inflammatory response syndrome (SIRS), sepsis and coagulopathy, and multi-organ failure. However, the exact mechanism behind this remains unknown.

Excluding well-known respiratory symptoms of SARS-CoV-2 infections, many patients reportedly develop gastrointestinal symptoms, including diarrhea, nausea, vomiting, and abdominal pain. In some cases, such symptoms manifested earlier than the classic fever and pulmonary manifestations of COVID-19.[5] Furthermore, numerous reports exist reporting hepatobiliary involvement in COVID-19 patients, as evidenced by abnormal liver function tests (LFTs), with their degree of elevation correlating with disease severity. Indeed, liver injury—manifesting as elevated LFTs with mild/moderate elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT)—in

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COVID-19 patients is reported to range from 14.8 to 53%.[6–8] These findings may be accompanied by slight elevations in total bilirubin and, in severe case, hypoalbuminemia.[9] With disease progression from mild to severe, LFTs correspondingly increase; AST and ALT elevations are reported to be present in approximately 56% of severely affected patients.[7] In short, varying degrees of liver pathology appear to be a salient feature of COVID-19 patients suffering from mild/moderate and severe infections.

Neutrophil extracellular traps (NETs)—released by neutrophils—have increasingly been attributed as major contributors to various liver pathologies, regardless of etiology, including sepsis, ischemic-reperfusion (I/R) injury, and portal hypertension.[10] Historically, neutrophils were confirmed to be the first immune effector cells recruited to sites of inflammation, where they mediate host defense through degranulation (antimicrobial release) and phagocytosis of offending microbes. In 2004, however, a novel third function was identified: NETs,[11] which are fibrous, DNA-based, web-like structures released by activated neutrophils by a unique form of programmed cell death termed as NETosis. NETs play vital roles in immunity by trapping and neutralizing microbes, preventing their dissemination. However, dysregulated NET production has been implicated as central to many immune-related diseases.[12] Indeed, NET secretion can damage normal cells in close enough proximity through various cytotoxic elements and, consequently, propagate proinflammatory responses.[12]

NET secretion is primarily induced by proinflammatory cytokines such as IL-1β released from macrophages secondary to inflammasome activation. Intriguingly, high levels of inflammasomes and its induced cytokines (i.e., IL-1β and IL-18) have been observed in COVID-19 patients, possibly implicating inflammasome activation as central to the SIRS, sepsis, and coagulopathy observed in severe COVID-19.[4] Considering the links between inflammasomes and NETs and the fact that neutrophilia is a cardinal feature of severe COVID-19 infections,[13–15] *we propose that excessive NET production by activated neutrophils participates in the pathogenesis of COVID-19, paying special attention to liver pathology.* Recently, the role of NETs in COVID-19 lung injury has been confirmed by histopathological examinations.[16,17] Numerous clinical trials are accordingly ongoing to evaluate the efficacy of NET-inhibiting or NET-lysing drugs in the treatment of COVID-19 patients. However, the pathomechanisms underlying systemic symptoms in severe patients still remain to be proven, which is the premise of our postulation. Future research aiming to substantiate NETs as important mediators of multi-organ pathology in severe COVID-19 could rationalize treatments for systemic COVID-19 symptoms.

Neutrophil Extracellular Traps: Structure and Function

Neutrophils are the most abundant circulating immune cells and characterize acute inflammation. Neutrophils contribute to host defense primarily via phagocytosis, generation of ROS, and degranulation. However, neutrophils have been implicated in the pathophysiology in a remarkable spectrum of diseases, including cardiovascular, inflammatory, autoimmune, metabolic, infectious, and septic conditions, through the production of NETs.[18] NETs are extracellular structures produced by a programmed form of neutrophil death termed as NETosis, resulting in the extrusion of neutrophil DNA and histones, which form a fibrous structure entrapping neutrophil granule proteins such as neutrophil elastase (NE) and neutrophil cytosolic proteins such as myeloid-related protein 14 (MRP14).[10,19] NETs play key roles in immune defense: histones, NE, and MRP14 exhibit bactericidal and antimicrobial properties; additionally, due to their fibrous structure, NETs occupy large amounts of space, they sequester bacteria in areas where the concentration of its antimicrobial components is high while simultaneously preventing microbial dissemination.[12,20–22]

Although vital in normal immunity, excessive NET formation can drive inflammation via damage-associated molecular patterns (DAMPs) and by host cell injury, particularly of the endothelium, through histones.[23] Additionally, the fibrous structure of NETs constitutes a platform to which red blood cells (RBCs), platelets, fibrinogen, and fibronectin can bind, precipitating thrombus formation.[24,25] Accordingly, NETs are well-established contributors to various disease processes, including vascular diseases such as atherosclerosis and hypercoagulability, metabolic diseases such as diabetes, autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, and, the focus of this manuscript, systemic inflammation in severe COVID-19.[18,26]

COVID-19 and Liver Disease

Numerous studies have assessed how the liver is affected during COVID-19, with abnormal LFTs mainly being characterized by mild elevations in serum AST/ALT. Two to eleven percent of patients with COVID-19 have pre-existing liver diseases, and 14–53% of cases feature elevated AST levels, with its magnitude of elevated correlating with disease severity.[7] Indeed, a study reported AST elevations in 62% of ICU-admitted patients, compared with 25% in patients who did not require ICU admission.[27] Accompanying abnormalities in serum bilirubin and, in severe cases, hypoalbuminemia, are also observed. Severe abnormalities in liver enzymes are also associated with a higher mortality.[7,9,19,28] Histopathological findings of liver autopsy specimens in patients who succumbed to COVID-19 demonstrated macrovesicular steatosis with mild lobular and portal inflammation. In some cases, vascular pathologies in the form of sinusoidal microthrombi were also observed.[8,17,29]

Collectively, these results implicate COVID-19 liver disease as being more prevalent in severe cases compared with mild cases. Since early on during the pandemic, concerns have been documented that patients with chronic liver disease may be increasingly susceptible to severe COVID-19 infections. These postulations are informed by several factors: (1) overlapping comorbidities associated with severe COVID-19 and liver diseases, including age, hypertension, and diabetes, and (2) the fact that chronic liver disease induces coagulopathy, a major risk factor for the development of severe COVID.[30] Lastly, the presence and degree of

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liver injury in COVID-19 has an important bearing on treatment. Mild COVID-19 liver injury is self-limiting and typically resolves without any specific treatment; in this case, therapy aims to actively treat primary disease. In contrast, acute liver injury in severe COVID cases—considered to be due to cytokine storms and circulatory collapse—requires liver protective drugs,[7] with simultaneous respiratory and circulatory support.[31]

The pattern and frequencies of LFT abnormalities are similar regardless of the presence of underlying chronic liver disease.[30] Furthermore, the mechanism behind SARS-CoV-2-induced liver disease remains uncertain but is likely multifactorial. In this article, we discuss (1) cytopathic effects from direct liver infection, (2) SIRS, and (3) in severe cases, hypoxic injury encompassing I/R injury and coagulopathy.

Direct Infection

As mentioned prior, LFT abnormalities mainly feature AST and ALT abnormalities. Intriguingly, AST and ALT elevations in COVID-19 patients do not correlate with inflammatory markers such as CRP or markers of rhabdomyolysis, hinting at the direct infection of the liver as being causal.[30] The angiotensin-converting enzyme 2 (ACE2) receptor has been established as the portal of entry of SARS-CoV and SARS-CoV-2 into cells. Following attachment to ACE2 to the spike (S) protein, cleavage of the S protein by the transmembrane serine protease 2 allows internalization of the virus by endocytosis.[32] The tropism of SARS-CoV-2 for the liver stems from the expression of ACE2 on hepatocytes and, in particularly high levels comparable to alveolar cells of the lung, on cholangiocytes (cuboid epithelial cells of the bile duct).[33,34] Furthermore, the isolation of SARS-CoV-2 RNA from stool samples of infected COVID-19 patients raises concerns about fecal-oral transmission.[19]

However, direct viral infection seems unlikely when correlated with the histopathologic evidence, since rather than the expected intracellular viral inclusions with concomitant lymphocytic infiltrates manifesting as piecemeal necrosis characteristic of viral hepatitis,[35] histopathology reports demonstrate microvesicular and macrovesicular steatosis. These observations were made in autopsies with SARS-CoV-2 as the only risk factor, distinguishing these lesions from hepatic steatosis due to pre-existing nonalcoholic fatty liver disease (NAFLD), which is regarded as an independent risk factor for poor COVID-19 prognosis.[36,37] Direct SARS-CoV-2 cytopathic effect-induced mitochondrial dysfunction has been implicated as potentially causing hepatic steatosis, a mechanism also seen in NAFLD; these findings have rationalized postulations that SARS-CoV-2 worsens pre-existing NAFLD-induced steatosis.[38] Alternatively, these characteristic histopathologic patterns of liver injury may also be due to pre-existing obesity and diabetes mellitus.[17]

These observations may also perhaps be due to the induction of endoplasmic reticulum (ER) stress upon the infection of the hepatocytes. This results in de novo lipogenesis.[38] In the setting of SARS-2-CoV, as well as the other coronaviruses, ER stress markers glucose-regulated protein 79 (GRP78) and GRP94 are elevated.[38,39] Lipogenesis may aid in viral replication and exocytosis from the cell. To this end, enhanced de novo lipogenesis has been suggested to provide the virus with the vesicular systems necessary for viral replication and exocytosis.[38] Other than ER stress, lipogenesis is also induced by the mammalian target of rapamycin (mTOR) pathway. Briefly, mTOR functions as the principal intracellular nutrient sensor. When nutrient levels are high, as suggested by the presence of hormones, growth factors, and glucose, mTOR activates protein synthesis and lipogenesis.[40,41] The mechanisms pertaining to mTOR-induced lipogenesis involve the activation of SREBP, a membrane-bound transcription factor, which binds specific response elements to upregulate the expression of genes pertaining to cholesterol and fatty acid synthesis.[40,42] In the context of COVID-19, direct infection of hepatocytes and elevated IL-6—due to the systemic cytokine storm seen in severe cases—could activate mTOR. Similar observations are seen in SARS-CoV-1 and MERS-CoV. mTOR, in turn, inhibits autophagy to prevent viral degradation in autophagolysosomes and enhances lipogenesis and protein synthesis.[43,44] The elevated protein synthesis induced by mTOR involves the activation of cap-dependent translation machinery, which is essential to coronavirus replication, which hijacks this machinery.[45] The de novo lipogenesis, as mentioned above, enhances viral replication and exocytosis. Accordingly, pre-existing mTOR hyperactivity—such as that seen in obese patients and diabetics—may, at least in part, explain the increased risk of severe COVID infections in these patients.[23,24]

However, since ACE2 is barely expressed on hepatocytes, further studies investigating SARS-CoV-2 directly infecting hepatic cells are required. Since cholangiocytic ACE2 expression is 20 times higher than hepatocytes and evidence of cholangiocyte proliferation has been observed in the setting of COVID, suggestions have been made of a compensatory proliferation of liver cells derived from the bile duct epithelium leading to an increased ACE2 expression in the liver.[19] High circulating levels of IL-6, a strong cholangiocellular mitogenic factor, may contribute to the proliferative response.[38]

The Cytokine Storm and SIRS

The majority of COVID-19 patients display little to no symptoms, while others suddenly deteriorate and develop severe fever and pneumonia, culminating in acute severe respiratory distress syndrome and ultimately death. Increasing evidence indicates that this presentation is probably secondary to a dysregulation of the innate immune response, resulting in a cytokine storm, systemic inflammatory response syndrome, and multi-organ failure, including the liver. Indeed, levels of numerous pro-inflammatory cytokines, such as IL-1β, IL-2, IL-6, IL-10, IL-17, TNF, and monocyte chemoattractant protein 1 are found to be elevated in patients.[46]

As previously intimated, the inflammasome may play a significant role in the immunopathogenesis of COVID-19, suggested by elevated serum levels of the inflammasome-related cytokines IL-1β and IL-18. Furthermore, the presence

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of inflammasome-related cytokines IL-1β and IL-18 in COVID-19 patients correlated with disease severity.[47,48] Inflammasome activation, regardless of the initiating stimulus, occurs in two steps: a priming stage, with an additional signal being required for full activation. The priming stage is constituted by the activation of pattern recognition receptors by DAMPs or pathogen-associated molecular patterns (PAMPs), which induce the activation of NF-κB promoting the gene expression of pro-IL-1β and pro-IL-18 and NOD-like receptor family pyrin domain-containing 3 (NLRP3).[49–51]

The second activation signal could be provided by several extracellular stimuli, including membrane damage, activation of ion channels, and reactive oxygen species (ROS), all of which promote NLRP3 oligomerization which, along with adaptor protein and caspase-1, forms the inflammasome and cleaves pro-IL-1β and pro-IL-18 into the active IL-1β and IL-18, respectively.[49,52,53] The last step of this process is the caspase-1-mediated cleavage of Gasdermin D (GSDMD), resulting in the formation of pores in the cell membrane to allow for the release of IL-1β and IL-18 as well as inducing cell death by pyroptosis, producing DAMPs.[54,55] IL-1β can also further activate the inflammasome (i.e., a feedforward mechanism).[3] This process collectively intensifies the immune response but, if not tightly regulated, can lead to massive infiltration of activated neutrophils with subsequent NETosis, activation of macrophages, and an exaggerated cytokine response leading to substantial tissue damage.[51,56,57]

In the context of coronaviruses, SARS-CoV directly activates the NLRP3 inflammasomes via its E protein and viroporin 3a. Since significant homology exists between SARS-CoV and SARS-CoV-2, there is likely significant overlap regarding pathophysiology. Both E protein and 3a protein behave as ion channels (viroporins): E protein associates with the ER membrane, causing leakage of Ca^{2+} into the cytosol; in contrast, viroporin 3a inserts at the cell membrane, promoting K^{+} efflux.[54,58] The consequent ionic imbalance, activation of various intracellular enzymes, and generation of ROS propagate mitochondrial and lysosomal membrane injury, which activates the NLRP3 inflammasome. Additionally, both E-protein and 3a proteins are found to activate NF-κB independently to drive the transcription of pro-IL-1β, proIL-18, and NLRP3.[52,53] Accordingly, the NLRP3 inflammasome has gained much attention as a potential target for therapy given its central role in the pathogenesis of severe COVID infection.

The liver plays an important role in immune defense, continuously being exposed to pathogens entering the body via the gut and containing the largest collection of fixed macrophages, called Kupffer cells, in the body.[59] Although Kupffer cells do not normally express ACE2 and, therefore, are not likely the target of direct SARS-2-CoV infection, monocyte-derived macrophages that replenish Kupffer cells in the setting of inflammation may provide a route of extension of the inflammatory stimulus.[38] Additionally, endothelial cells appear to play an important role. Alveolar endothelial cells are activated in the setting of inflammation, precipitating a hypercoagulable state and neutrophil activation with subsequent NETosis.[6] Therefore, widespread pyroptosis of macrophages not only in the lung but also in the liver followed by vigorous inflammation and hypercoagulability via endothelial cell activation is perhaps partly responsible for the lobular and portal inflammation, microvascular thrombosis, hepatic sinusoidal congestion, and necrosis observed in liver autopsies of deceased COVID-19 patients (readers are referred to[17] for more detailed descriptions). This could manifest clinically as hepatomegaly and elevated LFTs, with concomitant jaundice and hepatic encephalopathy, and elevated LDH. Serum LDH, in particular, is used as a marker of various inflammatory states and is significantly elevated in severe COVID-19 patients compared with those without the severe disease and, thus, can be used as a marker for severity and prognosis.[60–62]

Ischemic-Reperfusion Injury

The liver is highly vascular, and therefore, susceptible to hypoperfusion secondary to circulatory disturbances. Cardiac failure, respiratory failure, and circulatory shock are well established as mechanisms causing passive congestion and hypoperfusion of the liver resulting in centrilobular ischemic necrosis.[17,63,64] In the context of COVID-19, acute respiratory distress syndrome (ARDS) induces severe hypoxia that leads to ischemic liver damage characterized by steatosis and subsequent hepatocyte death.[31] This is followed by reperfusion characterized by neutrophil infiltration with the generation of ROS, all of which propagate further liver injury via lipid peroxidation and oxidation of DNA and proteins.[10,31]

Hypoxia independently imposes oxidative stress that promotes the generation of ROS, which have been identified as one of the key triggers of the inflammasome, evidenced by the chemical inhibition of ROS generation curbing inflammasome activation in response to several different stimuli.[65] The inflammasome-related cytokines IL-1β and IL-18 as well as DAMPs released by inflammasome-induced pyroptosis will exacerbate reperfusion injury, setting up a self-reinforcing loop of inflammation and tissue damage, and, as such, inflammasome-mediated inflammation is considered a potential therapeutic target to alleviate hepatic I/R injury in various surgical settings.[66] Such principles could potentially translate over to I/R injury in severe COVID-19 cases, where inflammasome inhibition can alleviate symptoms of hypoxic damage. Indeed, I/R resulting in hypoxic hepatitis secondary to anoxia induced by respiratory failure is seen in severe cases.[67]

The Potential Role of Nets in COVID/Liver Disease

In short, COVID-induced hepatic disease is likely to involve a combination of several mechanisms, including direct infection, cytokine storm and SIRS, thrombotic microangiopathy, and, in severe cases, hypoxic hepatitis. With increasing grades of disease severity, more of these pathomechanisms may get involved to cause liver injury, which manifests clinically as a mild-to-moderate rise of AST/ALT and a concomitant drop in serum albumin in severe cases.[7,63] The key players mediating each of these processes remain unelucidated, but the usual mediators, endothelial cell damage with neutrophils and platelets, likely play a role.[68]

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Identifying the major contributors to the pathogenesis of COVID-induced liver disease will likely have significant therapeutic implications, not only by revealing novel therapeutic targets but also perhaps by susceptible patient demographics and reliable prognostic biomarkers.

Recently, NETs have been implicated in the pathogenesis of severe COVID, characterized by a sudden, rapid deterioration of patients culminating as ARDS.[15,57,68] This hypothesis was supported by neutrophilia, elevated NET markers such as cell-free DNA and histones, and SARS-CoV-2 patient sera being able to induce NET formation.[69–71] Lastly, the degree of NET marker elevation correlates with pneumonia-associated lung injury.[72] The role of NETs in the pathophysiology of COVID is thought to be a primary result of vascular damage and the consequent exacerbation of inflammation with hypercoagulability, manifesting clinically as ARDS and multi-organ failure, including the liver.[21,73,74] Indeed, liver injury is a consistent feature of COVID-19, with histopathological evidence demonstrating patchy necrotic areas akin to NET-damaged livers.[75]

The liver functions as a frontline immune organ due to its unique blood supply, filtering the blood of any pathogens or PAMPs entering via the gut and subsequently eliciting appropriate immune responses. Furthermore, the liver has been reported to be the primary organ for bacterial sequestration which is thought to be fundamentally through the actions of two immune cells: Kupffer cells, the intrasinusoidal resting macrophages of the liver, through phagocytosis, and neutrophils, which are recruited to the liver in the setting of infection, via NET formation.[71,76] Indeed, apart from COVID, NETs have been well-established as key players in propagating liver injury in several pathologies, including alcohol-associated liver injury, portal hypertension in chronic liver disease, sepsis, liver transplantation, and even cancer.[10,18]

Potential Role of NETs in COVID-Induced Endotheliopathy

The role of NETs in multiorgan failure characterizing severe COVID-19 has been confirmed in several studies.[13,21,51,75] Both arterial and venous thrombosis are detected in COVID-19 patients, causing micro- and macrovascular thrombotic phenomena including acute coronary syndrome, deep vein thrombosis, and pulmonary embolism, with concomitant elevation in NET markers as well as neutrophil-platelet aggregates.[69,77] However, a current limitation in the study of NETs in COVID-19 is the lack of data on their role in mild-to-moderate COVID infections. COVID-19 patients, regardless of disease severity, demonstrate neutrophilia comprising of immature neutrophils, which are known to show increased NETosis at baseline.[68,75,78] As such, the role of NETs in mild-to-moderate disease may also exist. In the context of liver disease, elevated AST/ALT levels are also seen in non-severe COVID-19 patients.[9] With the current opinion that multiorgan manifestations of COVID-19 are secondary to a hyperinflammatory cytokine storm, why do moderate cases, which do not exhibit a hyperinflammatory state, exhibit elevated LFTs?

Intriguingly, several recent studies have suggested that accepting a cytokine storm as the principal mediator of COVID-19 may be premature. These postulations are based on the magnitude of serum cytokine elevations being insufficient to cause such symptoms.[79,80] To this end, a recent review article stated that organ-specific cytokines should be investigated.[68] In such a scenario, NETs may also be involved. Mounting evidence suggests that the pathophysiology of COVID-19 revolves around endothelial cell damage—so-called endotheliitis or endotheliopathy.[81–84] ACE2 is expressed on endothelial cells, and SARS-CoV-2 has consistently been detected within endothelial cells.[63,81,85] Endothelial cell activation and damage expose subendothelial collagen and other thrombogenic substances which attract platelets and neutrophils to collectively promote NETosis.[24] NETs can also directly activate endothelial cells as well as induce endothelial cell death through histones.[23,86,87] Therefore, since immature neutrophilia and elevated NET markers are observed in COVID-19 patients, their potential accumulation in the liver may represent a key trigger for endothelial cell injury and microvascular thrombotic events in COVID-19 patients in severe as well as non-severe cases.

Sepsis

Sepsis is characterized by a deadly inflammatory syndrome secondary to a dysfunctional immune response to infection. The liver is principally responsible for the clearance of bacteremia in the setting of sepsis through Kupffer cells by phagocytosis and neutrophils through NET formation. Liver sinusoidal endothelial cells (LSECs) upregulate TLR4 on their surface secondary to infection, facilitating retention of neutrophils within hepatic sinusoids by mediating neutrophil adherence to hyaluronan via CD44.[10,88] Hypoxia-induced injury of LSECs promotes the surface expression of p-selectin and von-Willebrand factor, which contributes to the recruitment and activation of neutrophils. The result of this is two-fold: NET production in the liver exceeds that of other tissues and, due to increased retention of NETs, the liver may also retain circulating NETs originally produced in other organs.[82,88]

While NET formation contributes significantly to bacterial sequestration and, therefore, plays a key role in immune defense, the high intrahepatic concentration of NETs may not only contribute to enhanced microbial sequestration but also, through its cytotoxic elements including but not limited to histones, NE, and cathepsin G, damage the liver and induce or exacerbate inflammatory responses and propagate further liver injury. Accompanying the inflammatory response in sepsis is disseminated intravascular coagulation (DIC), which is characterized by a consumption coagulopathy. NETs are thought to contribute to DIC through binding platelet, RBCs, and fibrinogen to precipitate thrombus formation.[24,57,89] Negatively charged DNA activates the intrinsic pathway of the coagulation cascade, and histones promote a hypercoagulable state by causing endothelial cell damage. Proteases within NETs, such as NE, are thought to activate the intrinsic and extrinsic pathways of coagulation.[90] Accordingly, research studying the significance of NET-mediated liver damage reports that the genetic inhibition of NET formation and various components of NETs greatly ameliorate symptoms of liver damage.[10,91]

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The endogenous stimulants of NET formation are believed to be histones, hypoxia, and high mobility group box protein 1 (HMGB1), which are released by irreversibly injured cells and function as DAMPs.[86,92,93] A study showed that treating neutrophils with exogenous histones or HMGB1 demonstrated a proportional increase in NET formation.[20] HMGB1 is a nonhistone protein normally linked to chromatin. Its translocation to the cytoplasm and subsequent extrusion into the extracellular space are mediated by high levels of NLRP3 inflammasome activity in immune cells, which simultaneously cause pyroptosis resulting in the production of more DAMPs to augment the inflammatory response.[54] Given the exaggerated NLRP3 inflammasome activity characteristic of severe COVID infection and its role in propagating the cytokine storm, inflammasome-mediated HMGB1 release into the extracellular space may induce excessive NET formation which further exacerbates the disease.

Conclusion and Perspectives

Collectively, we propose that NETs—observed to be elevated in COVID infection—play principal roles in liver injury in addition to acute lung injury. In support of our findings, serum NET markers are elevated in COVID-19 patients, which correlate with disease severity[70,71]; well-established inducers of NETosis, such as endothelial cells and proinflammatory cytokine IL-1β, are confirmed to play a major role in COVID-19 pathogenesis,[51,55,94] NETs play key roles in liver disease regardless of etiology,[10] and other, similar, immune-related diseases feature NETs,[12,26,95] and NETs are consistently detected within pulmonary,[75,96] renal,[16] and cardiac[97] microthrombi in COVID-19 patients.

Notably, the prognostic significance of LFT abnormalities in COVID-19 patients remains uncertain. However, since the onset of the pandemic, concerns have been raised about the susceptibility of chronic liver disease patients of COVID-19,[7,30] as these conditions share many of the same risk factors, including age, obesity, and diabetes. Additionally, advancing liver disease is associated with metabolic/endocrine derangements, immune dysregulation, and coagulopathy, all of which increase the risk of severe COVID-19.[30] Therefore, future work should aim to substantiate the prognostic value of LFTs in COVID-19 patients and, in keeping with the present discussion, evaluate potential relationships between liver abnormalities and NETs to inform therapeutic strategies to ameliorate this condition.

Further research is required to confirm the role of NETs in COVID-19. This holds potential therapeutic significance, as inhibition of NETosis could alleviate symptoms of ARDS and liver injury, as well as other organs because ARDS creates a hypoxic internal environment and liver injury manifests as metabolic disturbances detrimental to all organ systems. A likely challenge in the clinical application of such drugs will be the identification of appropriate patient demographics in which these drugs are indicated. Indeed, the potential clinical benefit of NET inhibition has to be counterbalanced with the possible deleterious effects these inhibiting key immune mediators could have on the patient trajectory. Nevertheless, given the central role of NETs in mediating lung, liver, kidney, and cardiac pathologies in COVID-19, numerous clinical trials are ongoing to evaluate the efficacy DNase-1 (dornase-alfa), a NET-lysing drug, in combating COVID-19.[98]

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None declared.

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