



# Understanding of COVID-19 Pathology: Much More Attention to Plasma Proteins

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## INTRODUCTION

The recent outbreak of the novel coronavirus infection (COVID-19) has produced a world-wide health problem (1). COVID-19 is extremely infectious and has high mortality rate in vulnerable individuals (2). On the other hand, there could exist a large percentage of infected patients, which exhibit mild symptoms or even no apparent symptoms. This situation could make surveillance control difficult to prevent the spread of infection efficiently. In the case of severely ill patients, the rapid and progressive development of the acute respiratory response syndrome (ARDS) is critical and often fatal, and associated with organ failure including cardiovascular disorders, lung embolism, acute kidney injury and DIC (1).

## COVID-19 AND THROMBOSIS/EMBOLISM

Clinical features in patients with severe COVID-19 include increases in D-dimers and fibrin product degradation as well as elevations in von Willebrand factor and soluble P-selectin, suggesting the existence of endotheliopathy along with platelet activation in COVID-19-associated coagulopathy (2–4). These findings were consistent with the fact that obesity, diabetes, and hypertension are the risk factors and major comorbidities for severe COVID-19 (4). Angiotensin converting enzyme-2, a receptor for the spike protein of severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2), is expressed on the surface of vascular endothelial cells as well as respiratory epithelial cells. Thus, vascular endothelial cells appear to be a target cell for SARS-Cov-2 infection and a primary site for invasion and replication that may induce abnormalities in endothelial cells, resulting in prothrombotic conditions in the vasculature. Findings from patient autopsies strongly suggest the presence of thrombus formation in lung vasculature as well as thromboembolism in the peripheral vessels (3–5). Thromboses of the coronary or carotid arteries have also been reported to result in sudden onset of fatal cardiovascular events (6, 7). Thus, in

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**Abbreviations:** COVID-19, coronavirus disease 2019; HRG, histidine-rich glycoprotein; IAIPs, inter  $\alpha$ -inhibitor proteins; MOF, multiple organ failure; NETs, neutrophil extracellular traps; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

addition to ARDS, the wide range of thromboses and embolism could be a fundamental pathophysiological component of severe COVID-19 (5).

## COVID -19 AND NEUTROPHIL EXTRACELLULAR TRAPS

Recent histopathological analyses of patients with COVID-19 have suggested that neutrophil extracellular traps (NETs) with web-like DNAs released from the cells bearing myeloperoxidase, neutrophil elastase and citrullinated histone 3 on DNA (8, 9), are involved in thrombus formation in the lung vasculature, suggesting a form of immunothrombosis (10, 11). NETs are thought to be induced by excessive activation of neutrophils resulting in an increase in cytosolic calcium and activation of protein kinase C, followed by activation of NADPH oxidase. The resulting production of reactive oxygen species is required for the translocation of neutrophil elastase and myeloperoxidase as well as citrullination of histone 3 and de-condensation of chromatin DNA. The immunothrombus starts from the adhesion of NETs on vascular endothelial cells, followed by platelet aggregation on the NETs along with fibrin deposition (12), suggesting a very crucial and important role for NETs in the development of immunothrombi.

The activation state of vascular endothelial cells is another factor that can predispose to the development of thrombosis (12). Endothelial cells expressing ACE-2, a receptor for the spike protein of SARS-Cov-2, can be activated after infection with SARS-Cov-2. Once the endothelial cells are activated, the interactions between endothelial cells and neutrophils, particularly the NETs, will be increased. The extracellular release of ROS and proteinases from neutrophils further facilitate the activation of endothelial cells, resulting in an increase in the expression of adhesion molecules on the surface of endothelial cells. Thus, dysregulated NETs on endothelial cells form a vicious cycle between them, followed by platelet aggregation on NETs. The coagulation cascade may initiate on the surface of aggregated platelets as well as on tissue factor-expressing endothelial cells. Finally, a series of events beginning with the NETs and the damaged vascular endothelial cells losing their anti-coagulant properties will result in immunothrombus formation (3, 4). Some groups have also reported the existence of NETs in interstitial and alveolar spaces beyond the vasculature in the lung parenchyma (13, 14), contributing to the development of ARDS. Therefore, the regulation of NETs has been suggested to be one of the directions for the treatment of COVID-19 (15, 16).

## INHIBITION OF NETS BY HISTIDINE-RICH GLYCOPROTEIN

Histidine-rich glycoprotein (HRG), a 75 kDa plasma protein primarily produced by the liver, has multiple functions. HRG regulates angiogenesis, coagulation/fibrinolysis, host defenses,

dead cell clearance and tumor growth (17). Recently, our group reported that a rapid decrease in plasma HRG in patients with septic disorders could trigger multiple organ failure (MOF) because HRG maintains circulating neutrophils in a resting state and protects vascular endothelial cells from excessive activation by several types of stimulants, thereby preventing NETosis and immunothrombosis (18). The loss of such homeostasis because of reductions in HRG may predispose to a cascade of events in septic patients resulting in respiratory failure, circulatory shock, renal failure and DIC (18, 19). We have already demonstrated that HRG inhibits NETs *in vitro* (20) and *in vivo* (18) and reduces ROS production (21). In addition, HRG protects vascular endothelial cells from excessive activation, inhibits the expression of adhesion molecules, inhibits HMGB1 release and suppresses cytokine production (22, 23). All these effects of HRG on vascular endothelial cells inhibit the interactions between vascular endothelial cells and neutrophils/platelets, maintaining the anti-thrombotic condition at the interfaces of the circulating blood and vascular wall (23). Moreover, HRG suppresses the intrinsic pathway of the coagulation cascade directly by inhibiting XIIa (24). A clinical study demonstrated that the plasma levels of HRG were lower in non-survivors than in survivors on the admission day in ICU patients (19). Therefore, plasma HRG could be a superior biomarker of sepsis compared with the current standard of care, which uses procalcitonin and presepsin as indicators of sepsis (19, 25).

## INTER $\alpha$ -INHIBITOR PROTEINS AS ANTI-SEPTIC PLASMA PROTEINS

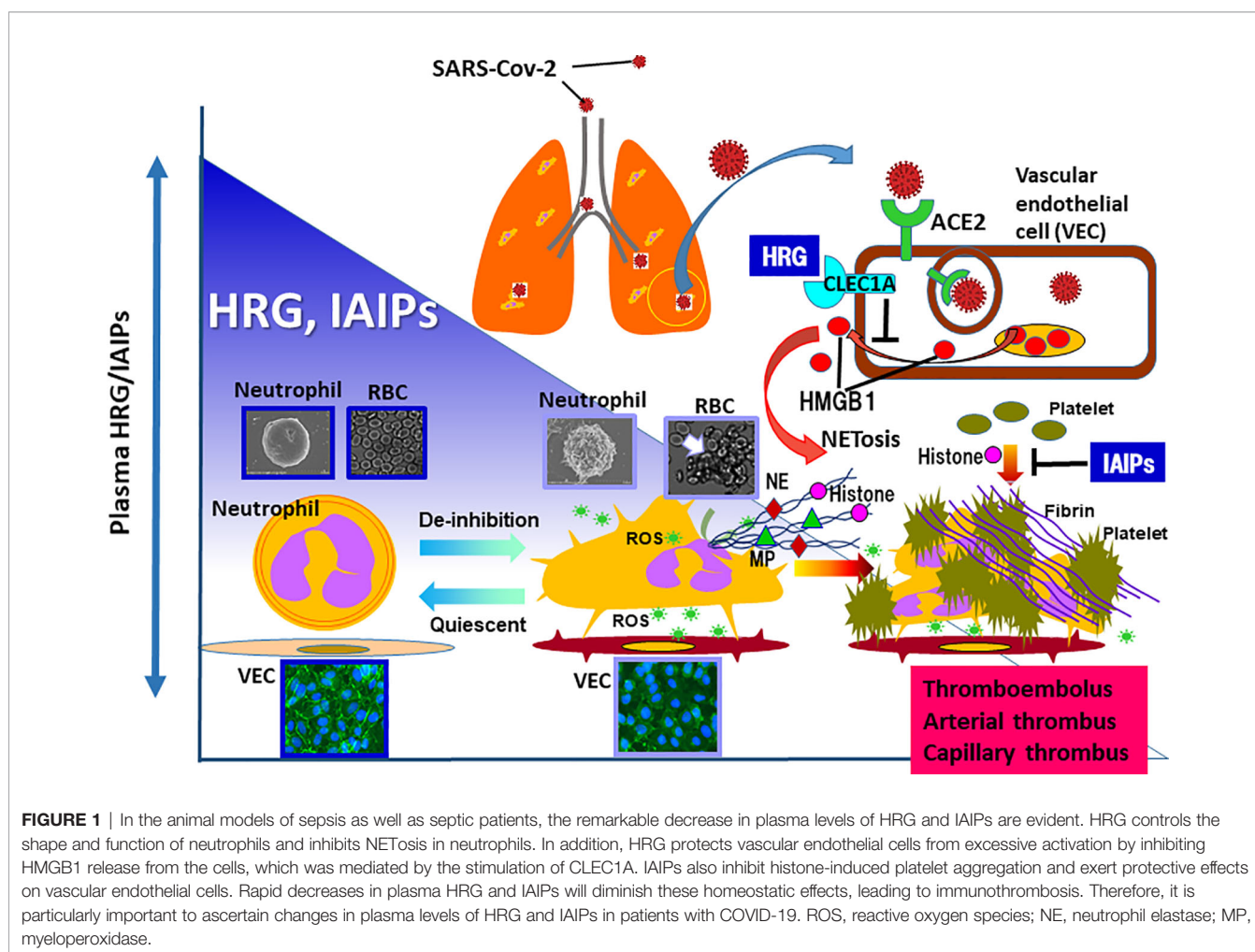
Inter  $\alpha$ -inhibitor proteins (IAIPs) are a family of structurally related serine proteinase inhibitors found in plasma in relatively high concentrations (around 500  $\mu$ g/ml). The major forms of these proteins in human plasma consist of two heavy chains and a single light chain called bikunin, covalently linked through esterification of chondroitin sulfate chain on bikunin. The minor forms contain different kinds of a single heavy chain coupled to bikunin in the same manner (26). IAIPs play a role in the regulation of a variety of responses including inflammation, wound healing, ovulation and cancer invasion/metastasis (26). In a neonatal animal model of sepsis, IAIPs decreased significantly after the induction of sepsis and supplementary therapy improved the lethality (27, 28). In addition, IAIPs have been suggested as both diagnostic and therapeutic agents in neonatal and adult septic patients (29, 30). IAIPs inhibit platelet aggregation induced by histone H3 (31) and suppress the spontaneous ROS production in neutrophils (32). Moreover, phenotypic analysis of IAIP knockout mice and the relationship between plasma levels of IAIPs and endothelial cell activation in septic patients imply that IAIPs ameliorate endothelial inflammation (33). These *in vitro* and *in vivo* findings could in part explain the some of the beneficial properties, by which IAIPs attenuate the effects of sepsis *in vivo*. Furthermore, it was reported that the decrease in plasma IAIPs corresponded

to the severity of Dengue virus infection and that the recovery from Dengue fever symptoms paralleled with the restoration of plasma IAIPs (34). Consequently, HRG and IAIPs could potentially represent two endogenous proteins that play key complementary overlapping roles to preserve intravascular blood cell and vascular endothelial cellular homeostasis in sepsis related disorders (**Figure 1**) (26, 32).

## HIGH MOBILITY GROUP BOX-1 AS AN IMPORTANT DAMP

High mobility group box-1 (HMGB1), a highly conserved nonhistone nuclear protein, plays a particularly important role as proinflammatory factor in the extracellular space through the stimulation of plural receptors: receptor for advanced glycation end products, toll-like receptor-4/2 (35). HMGB1 is released from not only necrotic cells but also many kinds of living cells under different conditions of stress

including hypoxia, ischemia, and stimulation by cytokines (36, 37). The neutralization of extracellular HMGB1 by specific monoclonal antibody (mAb) was reported to inhibit influenza virus (H1N1)-induced pneumonia and improve the survival of infected mice (38, 39). This antibody effect was additive to that obtained by the antiviral drug, peramivir (38). Therefore, the above-mentioned HRG regulation of HMGB1 translocation and HMGB1-induced cytokine production in vascular endothelial cells suggest that HRG (23) as well as anti-HMGB1 mAb (38, 39) could contribute to the maintenance of vascular endothelial cellular homeostasis through the control of HMGB1 resulting in inhibition of inflammation induced by influenza infection. Moreover, it was suggested that HMGB1 may facilitate NETs and NETosis (40). Based on these findings, it is speculated that HMGB1 may play a crucial role in the development of lung inflammation in COVID-19 (16, 41). In fact, Chen et al. (42) determined the plasma levels of HMGB1 in severe COVID-19 patients and found a significant elevation compared with those of healthy volunteers.



## CONCLUSION

These results from models of animal sepsis and the clinical findings suggest that decreases in HRG and IAIPs concentrations could represent excellent biomarkers to evaluate the severity of sepsis mainly caused by bacterial infections (25, 30). At present, we have no data on plasma levels of HRG or IAIPs in patients with COVID-19. However, it remains plausible that there might be a common process present in the cascade of ARDS/MOF irrespective of the etiology of sepsis, bacterial or viral. In addition to ARDS, venous thromboembolism or endovascularitis may occur in COVID-19 because recent analysis warned of the high incidence of these comorbidities in COVID-19 infections (3, 4). The findings of the protection of vascular endothelial cells by HRG (23) as well as IAIPs (33), and the prevention of platelet aggregation by IAIPs (31) and erythrocyte aggregation by HRG (43) support the functional role of these plasma proteins against venous thromboembolism. Therefore, considerable attention should be paid to the dynamic changes in the plasma levels of HRG and IAIPs, especially with regard to severe cases of

COVID-19, in order to delineate further the pathogenesis of this disorder.

## AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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