Stroke

ADVANCES IN STROKE

Role of Endothelial Cells and Platelets in COVID-Related Cerebrovascular Events

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In the past 2 years, the world, and health care specifically, has grappled with COVID-19 infections. It is now well established that infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has a vascular component and causes widespread endothelial dysfunction. Endothelial injury with SARS-CoV-2 infection causes pulmonary complications by reducing barrier properties, activating coagulation pathways and increasing the risk of disseminated intravascular coagulation.[1] The attachment of SARS-CoV-2 to ACE2 (angiotensin-converting enzyme type 2) receptors on endothelial cells, in the presence of TMPRSS2 (transmembrane protease serine 2), allows viral entry via clathrin-mediated endocytosis (Figure [A]) that activates a cascade of events causing endothelial injury and increased clot formation.[2] In addition to direct endothelial injury, SARS-CoV-2 binding to ACE2 decreases local beneficial effects of ACE2 on inhibition of the kallikrein-bradykinin system that further drives coagulation and inflammation (Figure [B] and [C]).[3] Given these known effects of SARS-CoV-2 infection on the vasculature, it is not surprising that SARS-CoV-2 also increases the rate of ischemic stroke and intracerebral hemorrhage, most notably in the young.[4,5] In addition, SARS-CoV-2 infection seems to disproportionally increase cryptogenic stroke and mortality suggesting the association between COVID-19 and stroke may be atypical with varying degrees of susceptibility.[6] Understanding the unique effects of SARS-CoV-2 infection on the cerebral endothelium is important for preventing and treating stroke in COVID-19 patients. In a novel study by Kaneko et al,[7] human endothelial cells and a 3-dimensional printed endothelialized model system were used to investigate ACE2 expression and underlying factors that affect cerebrovascular susceptibility to SARS-CoV-2 infection. In freshly obtained human brain and endothelium, mRNA levels of both ACE2 and TMPRSS2 were below detection. However, another ACE2 cofactor furin was found instead, suggesting protease-dependent membrane fusion (Figure [B]) as a means for SARS-CoV-2 entry into brain endothelium. Interestingly, when monolayers of human umbilical vein endothelial cells or human brain microvascular endothelial cells were subjected to high or low shear stress in the 3-dimensional model system, ACE2 expression increased substantially.[7] Further, when endothelial cells were grown in a 3D model that mimicked stenosis, ACE2 levels significantly increased compared with the nonstenotic area.[7] These findings may help explain localized endothelial injury and thrombosis with SARS-CoV-2 infection that leads to stroke—especially in young patients without typical stroke risk factors—but also why patients with co-morbidities that have preexisting vascular dysfunction such as diabetes and hypertension fair worse with COVID-19 infection.[8] Activation of the inflammatory cascade ("cytokine storm") that exacerbates endothelial dysfunction and blood-brain barrier permeability represents another means by which SARS-CoV-2 adversely impacts the cerebrovasculature that may be involved in COVID-19 stroke pathophysiology.[9]

ROLE OF PLATELETS IN COVID-19-RELATED CEREBROVASCULAR EVENTS

In addition to endothelial injury, COVID-19 infection is associated with increased thrombin generation and platelet activation that predisposes to venous, arterial, and microvascular thrombotic events, including

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Nonstandard Abbreviations and Acronyms

ACE2	angiotensin-converting enzyme type 2
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
TMPRSS2	transmembrane protease serine 2

stroke.[10] A hypercoagulability state is a major pathological event in COVID-19, and thromboembolism is a life-threatening complication of the infection. Platelets from COVID-19 patients aggregate faster and exhibit increased spreading on both fibrinogen and collagen.[11] They express higher levels of P-selectin basally and upon activation.[11] Aggregates of platelets and neutrophils, monocytes, and T-cells are significantly elevated in patients with COVID-19.[11] Platelet activation and platelet-monocyte interaction induce tissue factor expression in monocytes, which contributes to COVID-19 severity and mortality.[12] Platelet activation and monocyte TF expression were associated with markers of hypercogulability such as increased fibrinogen and D-dimers, which were increased in patients requiring mechanical ventilation or patients who died during hospital stay.[12] Platelets from severe COVID-19 patients induced TF expression in monocytes from healthy volunteers ex vivo, which was inhibited by platelet P-selectin neutralization or integrin α_{IIb}/β_3 blockade with abciximab.[12] Monocytes from severe COVID-19 patients displayed increased platelet binding and exaggerated tumor necrosis factor-α and interleuken-1β secretion in response to P-selectin and fibrinogen, suggesting that platelet-monocyte interaction amplifies inflammation and exacerbates thromboinflammation in COVID-19.[13] The thromboinflammatory storm in COVID-19 manifests clinically as acute respiratory distress syndrome, and in some patients as widespread thrombotic microangiopathy. Neutrophils, neutrophil extracellular traps, and complement play key roles in perpetuating fatal severe COVID-19.[14] SARS-CoV-2 can directly and indirectly induce neutrophil extracellular traps formation, which contributes to COVID-19 pathology by direct cytotoxic effects against epithelial and endothelial cells, formation of microthrombi and microvascular damage, and by perpetuating pathogenic autoantibody production.[14] Complement activation initially meant to contain the virus heightens inflammatory response and contributes to the severity of COVID-19.[14] Serum levels of endothelial cell adhesion molecules, including vascular cell adhesion molecule-1, intercellular adhesion molecule 1, and vascular adhesion protein-1 fractalkine are elevated in COVID-19 patients, with level of expression correlating with disease severity by promoting leukocyte adhesion to endothelial cells and thromboinflammation.[15] Low serum albumin correlates with SARS-CoV-2 disease severity and with D-dimer and thrombotic events.[16] Similar to sepsis, the reduction in albumin in COVID-19 infection is believed to be the result of suppression of albumin synthesis and loss due to capillary leakage. Reduced albumin within the microcirculation decreases oncotic pressure and causes tissue edema that, combined with capillary injury and dysfunction, exacerbate the pro-coagulant and thromboinflammatory state.[16] Finally, antiphospholipid antibodies have been reported in COVID-19 patients; however, it remains unclear whether antiphospholipid antibodies positivity contributes to increased risk of thrombosis in patients with COVID-19.[17]

Controversy remains as to which receptor mediates SARS-CoV-2 platelet interactions. Most, but not all, studies fail to detect ACE2 or TMPRSS2 on platelets and megakaryocytes.[18] ACE2 was not detectable by RNA sequencing, although SARS-CoV-2 mRNA fragments were detected in platelets from COVID-19 patients, suggesting that platelets may take-up SARS-COV-2 mRNA independent of ACE2.[11] In another study, platelets expressed ACE2 and TMPRSS2, and SARS-CoV-2 and its Spike protein directly enhanced platelet activation, which was inhibited by recombinant human ACE2 protein and anti-Spike monoclonal antibody.[19] Furthermore, Spike protein enhanced thrombosis formation in wild-type mice transfused with human ACE2 transgenic platelets, which this was not observed in animals transfused with wild-type platelets in vivo.[19]

Activated platelets release thromboxane A2, which further propagates platelet activation and aggregation.[11] Pretreatment with high-dose aspirin reduced platelet hyperreactivity in COVID-19 ICU patients.[11] Another study linked endothelial inflammatory activation to platelet-derived myeloid-related protein 8/14 and demonstrated that a $P2Y_{12}$ receptor antagonist reduced platelet-mediated proinflammatory effects on endothelium, suggesting that $P2Y_{12}$ may serve as a potential therapeutic target to reduce COVID-related endotheliopathy.[20] However, a subsequent randomized clinical trial did not show a benefit for adding a $P2Y_{12}$ inhibitor to a therapeutic dose of heparin in noncritically ill patients hospitalized for COVID-19.[21]

In summary, as illustrated in the Figure ([C]), SARCoV2 infection and entry into endothelial cells create a vicious cycle of endothelial injury and platelet-immune cell activation that lead to hyperpermeability, hypercoagulation, and thromboinflammation that increase the risk of ischemic and hemorrhagic stroke. Future studies aimed at understanding endothelial cell and platelet-immune cell interaction seem important for preventing COVID-19-related cerebrovascular events.

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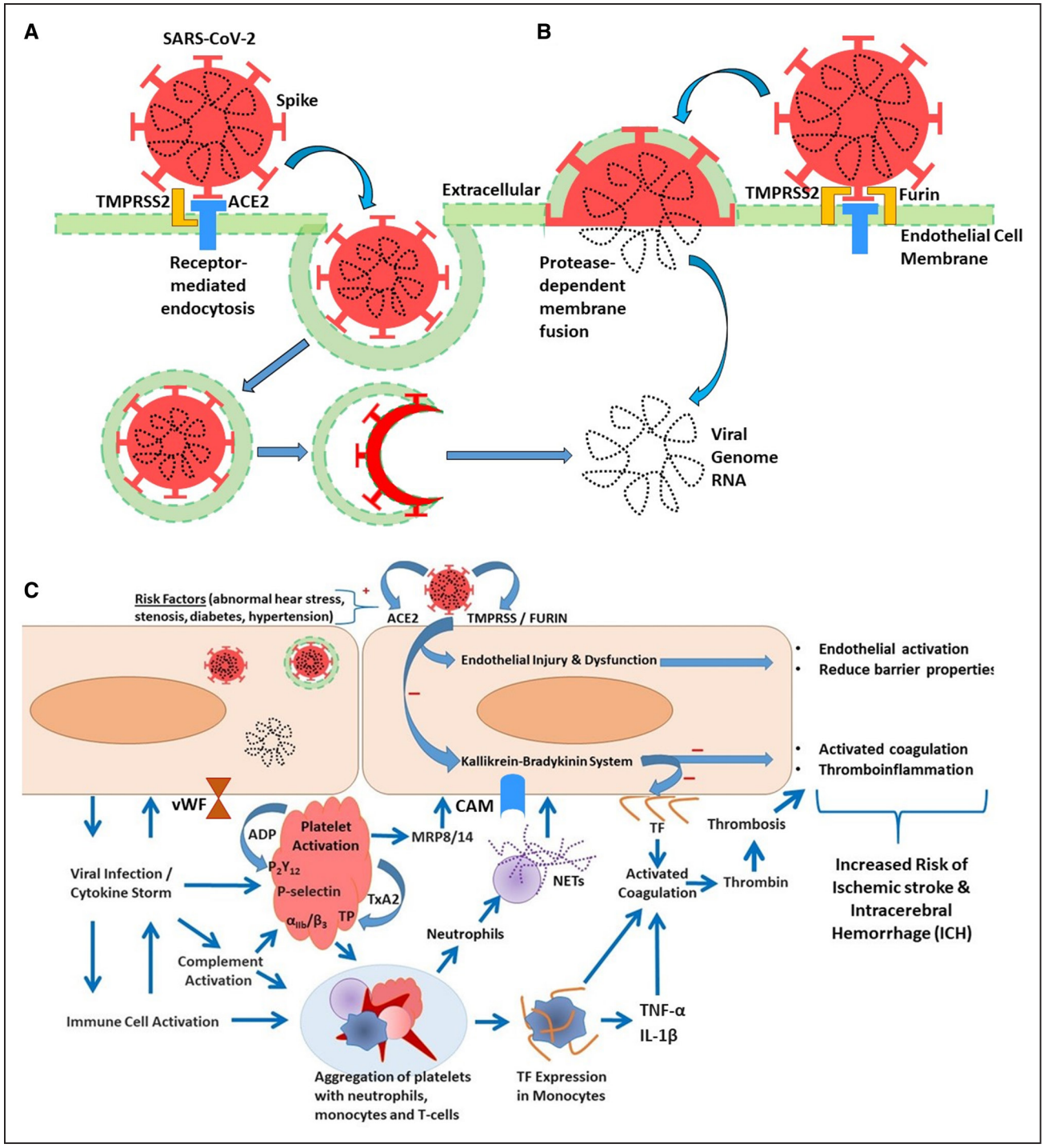

Figure. Mechanisms of severe acute respiratory syndrome coronavirus-2 (SAR-CoV2) entry and increased risk of stroke. **A**, ACE2 (receptor-mediated endocytosis in endothelial cells and cathepsin L-dependent activation. **B**, Protease (TMPRSS2 [transmembrane protease serine 2] and furin)-mediated membrane fusion and viral activation. **C**, Viral infection activates endothelial and immune cells, which secrete cytokines and chemokines that contribute to the cytokine storm and create a vicious loop that exacerbates endothelial cell activation and systemic inflammation, and lead to platelet activation and platelet-immune cell aggregation. Activated platelets and immune cells further contribute to the thromboinflammatory state by secreting cytokines, activating coagulation and through the formation of neutrophil extracellular traps. This is further exacerbated by complement activation. Platelet activation is amplified by ADP acting on the purinergic receptor $P2Y_{12}$ and thromboxane A2 (TxA2) acting on the thromboxane-prostanoid (TP) receptor. Activated endothelium expresses cell adhesion molecules (CAMs) and both endothelium and immune cells express tissue factor (TF), which activates coagulation, leading to thrombosis that further exacerbates endothelial injury and dysfunction, and heightens the risk of stroke.

ARTICLE INFORMATION

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