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SARS-CoV-2 cell entry receptor ACE2 mediated endothelial dysfunction leads to vascular thrombosis in COVID-19 patients

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**ABSTRACT**

Several studies have described unusually high incidence of vascular thrombosis in coronavirus disease-2019 (COVID-19) patients. Pathogenesis of the vascular thrombosis in COVID-19 is least understood for now and presents a challenge to the treating physicians. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative pathogen for COVID-19, has been shown to bind to angiotensin converting enzyme 2 (ACE2) protein in human epithelial cells which facilitates its entry in the organ and mediate tissue specific pathogenesis. For ACE2 mediated cell entry of the SARS-CoV-2, co-expression of one more protein—Transmembrane protease serine 2 (TMPRSS2) is essential. Existing studies suggested significant expression of ACE2 and TMPRSS2 in human vascular endothelium. Vascular endothelial dysfunction can potentially activate coagulation cascade eventually resulting in thrombosis. ACE2 has proven role in the maintenance of endothelial integrity inside the vessels. Existing in situ evidence for SARS-CoV-1 (the causative agent for SARS pandemic of 2002, which shared ACE2 as cell entry receptor) suggested that virus binding can downregulate ACE2, thus can induce endothelial dysfunction. Recently, in situ evidence has been presented that SARS-CoV-2 can infect cells in engineered human vascular endothelium, which can be effectively blocked by using clinical-grade recombinant human ACE2. Based on the circumstantial evidence present in the literature, we propose a SARS-CoV-2 cell entry receptor ACE2 based mechanism for vascular thrombosis in COVID-19 patients.

**Introduction**

Several studies have described high incidence of vascular thrombosis in coronavirus disease-2019 (COVID-19) patients [1–3]. Elevated levels of prothrombotic markers including D-dimer and fibrin degradation products (FDPs), thrombocytopenia, abnormalities in prothrombin time and partial thromboplastin time are among the frequent findings in COVID-19 patients [2] (Table 1), especially those with severe symptoms and in ICU admission [1–4]. Though, probability of vascular thrombosis remains relatively high in ICU admitted patients, in COVID-19 it has been found to be unusually high [1,3]. Pathogenesis of the vascular thrombosis in COVID-19 is rather obscure and hence management of such cases presents a considerable challenge to the treating physicians [1].

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative pathogen for COVID-19 has been shown to bind to angiotensin converting enzyme 2 (ACE2) protein in human epithelial cells, which facilitates its entry in the organ and mediate tissue specific pathogenesis [5,6]. ACE2 is also known as a receptor for SARS-CoV-1 (causative agent for SARS epidemic of 2002) and human coronavirus NL63 (HCoV-NL63) [7]. For ACE2 mediated cell entry of the SARS viruses, co-expression of one more protein—Transmembrane protease serine 2 (TMPRSS2) is essential [5]. In this article, we propose a SARS-CoV-2 cell entry receptor ACE2 based mechanism for vascular thrombosis in COVID-19 patients.
| Blood coagulation markers | Fan et al. | Chen et al. | Huang et al. | Peng et al. | Ming et al. | Shi et al. |
|---------------------------|------------|------------|-------------|-------------|-------------|-----------|
| Platelets (×10^9/L)       | 185        | 191.0 (156.8–230.0) | 164.5       | 191.0 (156.8–230.0) | 176 (91–292) | 187 (175.56–236.4) |
| Increased (%)             | 4.1 (%)    | 2.1 (0–11) | 11.1 (0–12) | 9.8 (0–12) | 11 (0–12) | 11.5 (0–12) |
| Prothrombin time (s)      | 12.5       | 11.5 (10–15) | 11 (0–12)  | 10 (0–12)  | 12.3 (9–14) | 11.5 (10–15) |
| Increased (%)             | 12.5 (%)   | 11 (%)     | 12.3 (%)   | 11 (%)     | 12 (9–14)  | 12.3 (%)   |
| Activated partial thromboplastin time (s) | 25.5 (15–33.3) | 28.7 | 27.1 (18.2) | 28.7 | 27.1 (18.2) | 25.5 (15–33.3) |
| Increased (%)             | 16 (6%)    | 16 (6%)    | 16 (6%)    | 16 (6%)    | 16 (6%)    | 16 (6%)    |
| D-dimer (μg/mL)           | 0.3 (0.2–0.4) | 0.2 (0.1–0.3) | 0.3 (0.2–0.4) | 0.2 (0.1–0.3) | 0.3 (0.2–0.4) | 0.2 (0.1–0.3) |
| Increased (%)             | 0.2 (%)    | 0.2 (%)    | 0.2 (%)    | 0.2 (%)    | 0.2 (%)    | 0.2 (%)    |

References provided as supplementary information. Parameters primarily indicating prothrombotic effects have been marked in red.

**Hypothesis**

SARS-CoV-2 binding to the cell entry receptor ACE2 downregulates receptor expression that in turn induces vascular endothelial dysfunction, which activates prothrombotic cascade and eventually leads to vascular thrombosis observed in COVID-19 patients.

**Evaluation of the hypothesis**

SARS-CoV-2 cell entry receptor ACE2 and entry associated receptor TMPRSS2 are co-expressed across various tissue and cell types in humans [5]. ACE2 expression is known in endothelial cells of human blood vessels and microvasculature [8,9]. Though, studies are limited, TMPRSS2 expression has been noted in human vascular endothelium [10,11]. ACE2 is an analogue of angiotensin converting enzyme (ACE)—a key regulator of renin angiotensin system regulating hemodynamic homeostasis in body [12]. An ACE/ACE2 balance appears to be crucial in maintaining the RAS and a dysregulation of this ratio is associated with vascular thrombosis [13,14]. An ACE/ACE2 balance is also critical for maintenance of endothelial integrity in vessels, a breach of which can potentially induce thrombosis [15,16]. Although, any SARS-CoV-2 specific evidence is lacking for now, existing in vitro studies for SARS-CoV1 and HCoV-NL63 binding on ACE2 in lung tissue indicate that virus binding downregulates ACE2 (but not ACE) creating ACE/ACE imbalance and thereby promotes tissue injury which potentially can activate prothrombotic cascades inside the vessels [7,17].

A lower ACE/ACE2 ratio in the vascular endothelium prevents prothrombotic cascade from activation by catalyzing degradation of angiotensin II to angiotensin 1–7. Angiotensin 1–7 also induces antithrombotic effects through binding to G-protein coupled Mas receptors [18]. Conversely, a higher ACE/ACE2 ratio will allow binding of angiotensin II to AT1 receptors which will induce vasoconstriction, inflammation, and eventually thrombosis [15,18]. SARS-CoV-2 mediated ACE2 downregulation in vascular endothelium also can activate the kallikrein–bradykinin pathway inducing platelet aggregation and leaking of the vessels which can further contribute to thrombosis [15,19].

Fig. 1 gives a schematic description of proposed mechanism for ACE2 mediated dysfunction of vascular endothelium leading to thrombotic incidences in COVID-19 patients.

**Empirical data**

Studies describing the effect of SARS-CoV-2 binding on ACE2 signaling in vessels are lacking. Knock-out of ACE2 gene in mice was found to induce vascular endothelial dysfunction leading to release of chemokines and pro-inflammatory markers [20]. ACE2 knock-out mediated release of chemokine like CCL2 and other pro-inflammatory cytokines and interleukins are known to induce platelet aggregation and adhesion to the vascular endothelium, which consequently leads to increased propensity for thrombosis [20,21]. Conversely, activated ACE2 has been found to protect against vascular thrombosis [14]. SARS-CoV-2 infection of vascular endothelium is mediated by ACE2 was proven by a recent study. Monteil et al provided in situ evidence that SARS-CoV-2 can infect engineered human vessels which can be prevented by application of clinical grade recombinant human ACE2 [8].

Recent studies noted remarkable similarities in clinical presentations in SARS and COVID-19 (apart from sharing of the virus entry receptor). Cases of vascular thrombosis were also reported in SARS patients [22]. SARS-CoV-1:ACE2 binding analogy to COVID-19 can explain the pathogenesis of vascular thrombosis with articulate coherence.

ACE2 is secreted in blood and expresses in the endothelium of the small vessels [9,23,24]. The histopathological examination of autopsy tissues in SARS, and now in COVID-19, showed presence of the virus...
and cellular damage in ACE2 expressing tissues [22,25,26]. Damaged tissue also displays presence of pro-inflammatory markers and cytokines, (e.g. C-reactive protein, D-dimer, ferritin and interleukin-6), which can induce thrombosis [1,22,27]. Viral inclusion structures, an accumulation of inflammatory cells associated with endothelium, as well as apoptotic bodies were evidenced in vascular endothelial cells of multiple organs in autopsied patients [28], indicating for the widespread endothelial dysfunction as the etiology for the vascular thrombosis in COVID-19 [29].

Consequences of the hypothesis and discussion

Widespread thrombosis, more specifically in microvasculature, was found present in majority of the autopsied patients [30]. In some cases, thrombosis was observed despite full anticoagulation and regardless of timing of the disease course of the duration of onset of the disease, which indicated that it could be a key pathology in COVID-19 [30]. Thrombus rich in megacaryocytes and platelets was detected in lung, and many other viscera like liver, heart, kidney, and brain, and also in skeletal tissue like bone [30]. Pulmonary vessels were found most severely affected where distinctive vascular features, consisting of severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes, and perivascular T-cell infiltration were observed [31]. Presence of vascular thrombosis was associated with multi organ injury and higher fatality. Formations of clots were usually high in patients who were critical and admitted in ICU. Clinically, early appearances of thrombotic symptoms predicted a more severe course of disease [30]. Uniquely, purple or red lesions in toes and skin rashes have been reported in some patients [27], which are supposedly caused by inflammation of the dermal micro-vehicles and are complement system mediated [25].

The optimum conditions leading to thrombosis like presence of proinflammatory markers, reduced clotting time, activated platelets and fibrin degradation products, and complement cascades were observed in the blood samples of COVID-19 patients (Table 1). Existing studies for SARS and MERS, and now emerging evidence from autopsy studies in COVID-19 provide clear indications that increased thrombosis may have contributory roles in thrombotic incidences in COVID-19 patients. Apart from direct SARS-CoV-2 inflicted tissue injury, viral protein component or viral receptor, virus-receptor complex [37], or auto-immune reactions against viral protein component or viral receptor, virus-receptor complex [37], may have contributory roles in thrombotic incidences in COVID-19 patients.

Currently, the therapeutic approaches which are being suggested to prevent vascular thrombosis in COVID-19 patients are primarily directed at inhibition of coagulation cascade at certain steps or preventing thrombus formation [38]. Chief anticoagulants which are being used are low molecular weight heparin (LMWH) as prophylaxis and treatment, unfractionated heparin (UH) where disseminated intravascular coagulation (DIC) is threatened, or fibrinolytics like tissue plasminogen activation (tPA) inhibitors (as a salvage therapy). Anti-thrombotic drugs primarily being used are that inhibiting platelet aggregation and glycoprotein IIb/IIIa receptor [38]. Existing evidence for the antiviral properties of heparin against coronaviruses like SARS-CoV-
1 and human coronavirus NL63 (HCoV-NL63) suggested comparative benefits of using this (however any direct proof for the antiviral property of heparin against SARS-CoV-2 is lacking for now) [39,40].

Targeting SARS-CoV-2 binding to ACE2 receptor may potentially prevent endothelial dysfunction and consequent initiation of thrombotic cascade [41], however, application of this approach in COVID-19 is scarce in literature. In vitro/in vivo testing of the molecules which can counter SARS-CoV-2 mediated downregulation of ACE2 and consequently endothelial dysfunction, e.g., clinical grade recombinant human ACE2 [8] or an ACE2 activator [42] may signify therapeutic implications of our hypothesis. (Diminazene aceturate (DIZE), an ACE2 activator [42], is currently being used as an anti-protozoan veterinary drug (an active ingredient of ‘Berenil’), however, in clinically effective doses it has serious toxicity risk for vital organs such as liver and kidney, and more particularly for the central nervous system (CNS) and heart. Adequate safety testing, and determination of safe dose, in human subjects, will be necessary, before it can be repurposed in COVID-19 patients.)

Based on the circumstantial evidence present in the literature, we propose that thrombosis in organ vasculature and dermal micro-vessels in COVID-19 are primarily caused by virus binding mediated dysregulation of ACE2 signaling in vascular endothelium and consequent cellular injury and activation of hemostatic factors. However, contributory role of other prothrombotic factors (related to both virus and host) cannot be denied.

Further in vivo/in vitro studies investigating SARS-CoV-2 binding influence on ACE2 signaling and consequently on molecular cascade involved in clotting in vascular endothelium will be necessary to establish ACE2 based mechanisms in thrombotic incidences in COVID-19.

Authors contribution
A.K. designed research. A.K., R.K.N., and C.K. analyzed data. A.K., M.A.F., M.K.K., and V.P. wrote the paper.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110320.

References
[1] Bakieli B, Madhavan MV, Jimenez D, Chuch T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. J Am Coll Cardiol 2020;75(23):2950–73.
[2] Ranucci M, Ballotta A, Di Dedda U. The procoagulant pattern of patients with COVID-19 and human coronavirus NL63 (HCoV-NL63) suggested comparative benefits of using this (however any direct proof for the antiviral property of heparin against SARS-CoV-2 is lacking for now) [39,40].
[3] Nasucci M, Ballotta A, Di Dedda U. The procoagulant pattern of patients with COVID-19 and human coronavirus NL63 (HCoV-NL63) suggested comparative benefits of using this (however any direct proof for the antiviral property of heparin against SARS-CoV-2 is lacking for now) [39,40].
[4] Makris M, Karabinis A, Kontoyiannis D, et al. COVID-19: the vasculature unleashed. Nat Rev Immunol 2020;21:1–3.
[5] Zhang Y, Cao W, Xiao M, et al. Clinical and coagulation characteristics of 7 patients with COVID-19 acute respiratory distress syndrome [published online ahead of print 17 April 2020]. J Thromb Haemost. 2020;18(4):844–7.
[6] Zhang H, Zhou P, Wei Y, Yue H, Wang Y, Hu M, et al. Histopathologic Changes and implications of our hypothesis. (Diminazene aceturate (DIZE), an ACE2 activator [42], is currently being used as an anti-protozoan veterinary drug (an active ingredient of ‘Berenil’), however, in clinically effective doses it has serious toxicity risk for vital organs such as liver and kidney, and more particularly for the central nervous system (CNS) and heart. Adequate safety testing, and determination of safe dose, in human subjects, will be necessary, before it can be repurposed in COVID-19 patients.)

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[1] Bakieli B, Madhavan MV, Jimenez D, Chuch T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. J Am Coll Cardiol 2020;75(23):2950–73.
[2] Ranucci M, Ballotta A, Di Dedda U. The procoagulant pattern of patients with COVID-19 and human coronavirus NL63 (HCoV-NL63) suggested comparative benefits of using this (however any direct proof for the antiviral property of heparin against SARS-CoV-2 is lacking for now) [39,40].
[36] Previtali E, Bucciarelli P, Passamonti SM, Martinelli I. Risk factors for venous and arterial thrombosis. Blood Transfus 2011;9(2):120–38. https://doi.org/10.2450/2010.0066-10.

[37] Amiral J, Vissac AM, Seghatchian J. Covid-19, induced activation of hemostasis, and immune reactions: can an auto-immune reaction contribute to the delayed severe complications observed in some patients? Transfus Apher Sci 2020;59(3):102804. https://doi.org/10.1016/j.transci.2020.102804.

[38] McFadyen JD, Stevens H, Peter K. The emerging threat of (Micro)thrombosis in COVID-19 and its therapeutic implications. Circ Res 2020. https://doi.org/10.1161/CIRCRESAHA.120.317447.

[39] Lang J, Yang N, Deng J, et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. PLoS ONE 2011;6(8):e23710. https://doi.org/10.1371/journal.pone.0023710.

[40] Milewska A, Zarebski M, Nowak P, Stocek K, Potempa J, Pyrc K. Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells. J Virol 2014;88:13221–30. https://doi.org/10.1128/JVI.02078-14.

[41] Jung F, Krüger-Genre A, Franke BP, Hufert F, Küpper JH. COVID-19 and the endothelium. Clin Hemorheol Microcirc 2020;75(1):7–11. https://doi.org/10.3233/CH-209007.

[42] Castardeli C, Sartório CL, Pimentel EB, Forechi L, Mill JG. The ACE 2 activator diminazene aceturate (DIZE) improves left ventricular diastolic dysfunction following myocardial infarction in rats. Biomed Pharmacother 2018;107:212–8. https://doi.org/10.1016/j.biopha.2018.07.170.