Endothelial cells and blood vessels are major targets for COVID-19-induced tissue injury and spreading to various organs

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Abstract
The coronavirus disease 2019 (COVID-19) infected so far over 250 million people and caused the death of over 5 million worldwide. Aging, diabetes, and cardiovascular diseases, conditions with preexisting impaired endothelial functions predispose to COVID-19. While respiratory epithelium is the main route of virus entry, the endothelial cells (ECs) lining pulmonary blood vessels are also an integral part of lung injury in COVID-19 patients. COVID-19 not only affects the lungs and respiratory system but also gastrointestinal (GI) tract, liver, pancreas, kidneys, heart, brain, and skin. Blood vessels are likely conduits for the virus dissemination to these distant organs. Importantly, ECs are also critical for vascular regeneration during injury/lesions healing and restoration of vascular network. The World Journal of Gastroenterology has published in last two years over 67 outstanding papers on COVID-19 infection with a focus on the GI tract, liver, pancreas, etc., however, the role of the endothelial and vascular components as major targets for COVID-19-induced tissue injury, spreading to various organs, and injury healing have not been sufficiently emphasized. In the present article, we focus on these subjects and on current treatments including the most recent oral drugs molnupiravir and paxlovid that show a dramatic, significant efficacy in controlling severe COVID-19 infection.

Key Words: Endothelial cells; Impaired endothelial function; Blood vessels; SARS-CoV-2; COVID-19; Cytokine storm

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Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has enormous health care and economic impact on the entire world - infecting more than 250 million people in 213 countries and territories, causing death of more than 5 million (as of November 1, 2021). We comment here on some outstanding papers on COVID-19 published in World Journal of Gastroenterology and reviewed the important role of endothelium and blood vessels in COVID-19 infection. Endothelial cells and blood vessels are both the targets and a conduit for the spread of severe acute respiratory syndrome coronavirus 2 and play a critical role in COVID-19-induced tissue injury and dissemination to various organs. Pre-existing endothelial impaired function could make endothelial cells more sensitive to COVID-19 or at least COVID-19-induced impairment might be synergistic with pre-existing impairment. That could be one contributing factor explaining why older or diabetic patients have more severe responses to infection, since these conditions are already impacted impaired endothelial function.

BIOGRAPHY

Andrzej S Tarnawski, MD, PhD, DSc (Med), AGAF, FACG: Received MD degree, PhD (pathology) and DSc (gastroenterology) from the University Medical School, Krakow, Poland, and became Associate Professor & V-Chair, Dept of Gastroenterology at that University. After completing GI fellowship at the University of Missouri, Columbia, MO, United States he joined the University of California, Irvine, USA as Associate Professor (1982-1986) and full Professor (1986-present). He served as: V-Chair and Associate Chair, American Gastroenterological Association (AGA)/EGD 1997-1999 and 2008-2010; Scientific Director, Shimoda Symposia on Mucosal Defense in Japan (8 times); Chair, Research Fora DDW/AGA annual meetings (1996-2011); Chair, Pasteur Institute Euroconference and Chair/Co-chair of 68 other International Symposia. Publications, presentations & grants: 373 full, peer reviewed publications (Lancet, Nature Med, JCI, Gastroenterology, Gut, PNAS, PASEB J, Am J Pathol, Cellular Mol Gastro Hepatol, Am J Physiol, Am J Gastroenterol, Endoscopy, Cell Signal, Cells, and others); 20 book chapters; 533 presentations at international & U.S. meetings; 20 peer reviewed funded grants (NIH, VA Merit Review 1984-present), 4 US patents. Clinical and Research interest: Injury and protection of GI mucosa; cellular and molecular mechanisms of gastric, duodenal and esophageal healing-role of growth factors, signaling pathways, angiogenesis, NSAIDs, prostaglandins and Helicobacter pylori toxins; aging gastric mucosa; confocal endomicroscopy and molecular imaging; gene therapy. Awarded prestigious academic honors (e.g., Glaxo Intl. Res. Award, Athalie-Clarke Award, Merenitus Medal Award, Peregrinator of Science Awards, Andre Robert’s Distinguished Award, Notable Biomedical Research Investigator Award). Memberships: AGA (Fellow), Am. College of Gastroenterology (Fellow), Brit. Soc. of Gastroenterology, Japanese Soc. of Gastroenterology (Honorary), Hungarian Soc. of Gastroenterology (Honorary), Am. Soc. for Investigative Pathology, Association of Am. Physicians (by election) and others. Editorial Boards - 6 scientific journals. Sixteen of his former trainees hold academic positions in US Medical Schools (4 being Chairs of Departments). Twenty of his former international trainees and/or associates hold academic positions abroad (France, Germany, Hungary, Japan, Poland, Sweden, Switzerland) (Figure 1A).

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has had enormous health care and economic impact on the entire world - infecting more than 250 million people in 213 countries and territories, causing more than 5 million deaths (as of November 1, 2021). Its enormous magnitude is also reflected by an unprecedented number of publications related to COVID-19 so far approximate 210294 recorded in PubMed; 254358 recorded on PMC, and 3215 clinical trials just in 24 mo. These are staggering numbers compared to 47305 publications recorded on PubMed on Helicobacter pylori (H. pylori) – the world’s most prevalent GI infection - published in about last 40 years.

COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is highly infectious and transmitted by aerosol droplets. Therefore, it is not surprising that the respiratory tract including the lungs is the main affected organ by COVID-19 infection that leads to respiratory failure, hypoxia, multiorgan system failure and death. Numerous studies showed that COVID-19 not only affects the lungs and respiratory system but also the gastrointestinal tract (GI), liver, pancreas, kidneys, heart, brain, and skin[1-5]. SARS-CoV-2 RNA was detected in stool or rectal swabs in 34%-59% of infected patients[6]. The viral loads from stool samples peaked 2-3 wk after symptom onset and in some patients were detectable even after viral loads in the respiratory and/or sputum samples were not detectable[6]. The presence and persistence of viral RNA in the stool suggest the potential for enteric infection of SARS-CoV-2. This contention is supported by a study demonstrating that the GI tract is an alternative route for COVID-19 infection in the rhesus monkey model[7]. In that study, the authors showed that intranasal or gastric inoculation with SARS-CoV-2 induced infections and pathologic changes not only in respiratory tissues but also in digestive tissues[7]. In a recent letter to the World Journal of Gastroenterology (WJG) editor[8], Sica et al[8] contended that GI and hepatic involvement are the most common presenting symptoms of COVID-19 and multisystem inflammatory syndrome recently described in children and adolescents. This syndrome can lead to shock and multiple organ failure requiring intensive care[9].

Risk factors for COVID-19 severity include aging and comorbidities such as coronary artery disease, chronic kidney disease, hypertension, obesity, and diabetes[10-12], all of which exhibit preexisting endothelial dysfunction. However, the potential role of endothelial/vascular components as critical target sites for COVID-19-induced tissue injury and spreading to various organs, and the role of preexisting endothelial function impairment, e.g., in aging or diabetes – conditions that facilitate...
COVID-19 infection have not been sufficiently elaborated on. In the present article, we focus on these topics anticipating that providing a detailed information on endothelial cells (ECs) and vasculature in COVID-19 as critical targets may afford a better insight into the pathomechanism of this disease and add additional new therapies.

The SARS-CoV-2 virus spreads from its primary infection site (respiratory tract) to more distant organs indicating the involvement of ECs and blood vessels for disseminating infection. This contention is supported by some studies demonstrating the presence of SARS-CoV-2-like particles in ECs in several tissues e.g., lung, kidneys, brain, and skin and observation that the clinical course of COVID-19 may include vascular complications such as thrombosis of blood vessels and thromboembolism[3,5,13-16].

The WJG has published in the last two years over 67 outstanding papers related to COVID-19 infection with a focus on GI tract and liver. These papers - original papers, retrospective studies and review articles on the pathophysiology, mechanisms, and clinical aspects and manifestations of COVID-19 related diseases of the digestive system including GI tubular system, liver, pancreas provided important information for the gastroenterologists, hepatologists, surgeons, researchers, pharmacologists, and clinicians. These papers provide information on the mechanisms of COVID-19 related tissue damage; the effects of immunosuppression in patients with inflammatory bowel disease and chronic liver disease; and the impact of COVID-19 on GI emergencies, endoscopy, diagnosis and treatments. These WJG articles were frequently viewed on the WJG website and cited in high-impact journals. We wish to point out one important paper by P. Samantha and AR Ghosh: “Environmental perspectives of COVID-19 outbreaks: A review” published in World J Gastroenterol. 2021 Sep 21;27(35):5822-585”[17]. In this paper the authors provided extensive information from an environmental perspective on the origin and current status of COVID-19[17] and summarized the geographical distribution of COVID-19 around the world including specific countries. They also elaborated on the details of coronavirus genus, species and receptors, virus susceptibility and incubation period, and summarized SARS-CoV-2 pathogenesis, the role of angiotensin-converting enzyme 2 (ACE2), the longevity of SARS-CoV-2 virus in the environment, meteorological influences, air quality and social impact. They emphasized that aging, cardiovascular diseases and diabetes predispose to COVID-19. The authors stressed that while drugs such as remdesivir, tocilizumab, lopinavir-ritonavir, azithromycin, etc., are used in COVID-19 patients these drugs do not induce full recovery. The statement that there is no truly effective drug aimed at the causative agent, SARS-CoV-2 is no longer valid. On November 4 and 5, 2021 the released results of most recent clinical trials for COVID-19 treatments demonstrated that oral drugs inhibiting viral replication - Molnupiravir (Merck), and Paxlovid (Pfizer) showed very impressive efficacy in controlling severe COVID-19 infection. The interim analysis of the latter drug showed a dramatic approximate 90% reduction in risk of - hospitalization or death from COVID-19 compared to placebo in patients treated within three - five days of symptom onset. Most likely the vascular component of the disease was important part of this dramatic reduction.

Regarding COVID-19 pathomechanism, the potential role of endothelial and vascular components as critical target sites for COVID-19-induced tissue injury and spreading to various organs and the role of preexisting endothelial function impairment, e.g., aging gastropathy has not been sufficiently emphasized. In this editorial article, we focus on the role vascular endothelium and blood vessels in COVID-19 infection (Table 1).

Increasing evidence suggests the essential role of endothelium and vasculature, in addition to the epithelial cells, in COVID-19 infection as a critical targets for SARS-CoV-2 and the resulting cytokine storm, and as the main effector for the pro-inflammatory and pro-coagulant state in COVID-19 patients[18,27-30]. Focus on ECs and vasculature in COVID-19 may also add additional insight into COVID-19 injury, its healing and tissue regeneration, and new therapies that impact endothelium and the blood vessels.

Although SARS-CoV-2 primarily targets the respiratory and alveolar epithelium, the high incidence of vascular complications in COVID-19 patients suggests that impaired function of ECs, which line the blood vessels and microvessels, may be critical factor in COVID-19 progression. SARS-CoV-2 causes endothelial dysfunction and thrombosis by two potential mechanisms: by directly infecting the endothelium, and disrupting its anti-thrombogenic and barrier properties, or indirectly by unleashing a local cytokine storm and systemic inflammatory response that results in endothelial injury (Table 2). Most likely, both these scenarios are in play in COVID-19.
**Table 1 COVID-19 and endothelium/blood vessels**

| COVID-19 and endothelium/blood vessels |
|----------------------------------------|
| Endothelium and blood vessels are integral parts of COVID-19-induced tissue injury. Their injury is likely due to either direct viral infection and/or cytokine storm triggered by the infection of adjacent epithelial cells and inflammatory response[18]. |

Blood vessels are critical for virus dissemination to distant organs.

Preexisting-impaired endothelial function, e.g., in aging or diabetes are likely predisposing factors COVID-19. Our studies demonstrated that aging gastric mucosa has increased susceptibility to injury and prominent EC abnormalities (decreased VEGF, NGF and impaired mitochondrial function)[19-21]. ECs are critical for vascular regeneration (through angiogenesis and vasculogenesis) during injury/lesions healing and therefore are essential for the delivery of oxygen and nutrients to the healing site[22,23].

Several growth factors e.g., NGF, IGF-1, HGF and BMD-stem cells may facilitate tissue regeneration in the healing phase[20,24,25].

Long-term effects of COVID-19, its vaccines and treatment on endothelium and vasculature remain to be determined.

Recently, new oral drugs inhibiting viral replication–Molnupiravir (Merck) and Paxlovid (Pfizer) showed significant efficacy in controlling severe COVID-19 infection by inhibiting viral replication. The interim analysis of the latter drug showed an 89% reduction in risk of COVID-19-related hospitalization or death from any cause compared to placebo in patients treated within three-five days of symptom onset[26].

**Table 2 Scenarios by which SARS-CoV-2 elicits endothelial damage**

| Scenario A: SARS-CoV-2 infection | Scenario B: Cytokine storm |
|----------------------------------|---------------------------|
| SARS-CoV-2 infects and replicates within vascular ECs and new virus particles are released into the blood vessel. These virions can infect neighboring cells or are carried to distant organs via circulation | ↑ IL-6, IL-1β, and TNFα release (cytokine storm) → endothelial damage |
|                                  | ↑ vascular permeability → plasma extravasation |
|                                  | ↑ vWF & FVIII (promote clot formation) and ↑ PAI-1 (inhibits clot lysis) → hypercoagulation |

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IL: Interleukin; TNF: Tumor necrosis factor; vWF: von Willebrand factor.

**Endothelium in normal and pathological conditions. Role in homeostasis, tissue repair and healing**

The endothelium is a key player in vascular homeostasis[29,31-33]. ECs are critical for supplying oxygen and other nutrients to all cells and tissues, and are involved in coagulation and the generation of vasoactive substances, prostanoids, hormones and growth factors[33-38]. The unstimulated vascular endothelium is normally impermeable and acts as a selective barrier regulating exchange of fluids, nutrient delivery and waste removal while preventing entry of pathogens and harmful substances into the tissues. Microvessels consist of a single layer of thin (approximate 0.5-1 μm) ECs and occasional adherent cells such as pericytes[34-38]. The endothelial barrier between neighboring ECs formed by prominent tight junctions prevents diffusion between cells. ECs act as a barrier between blood and the interstitiumal, and regulate various physiological processes such as angiogenesis, inflammation, and immune response[31,35,36]. The endothelium contains special vesicles - Weibel-Palade bodies, which store various factors that regulate blood coagulation and leukocyte recruitment and extravasation such as von Willebrand factor (vWF), P-selectin, chemokines, interleukin-8, and eotaxin-3; endothelin-1, angiopoietin-2 and osteoprotegerin[39-42].

In response to local stimuli, ECs secrete endothelin and leukotriene C4 (potent vasoconstrictors), nitric oxide (NO) and prostacyclin (PGI2) (vasodilators) and empty the contents of the Weibel-Palade vesicles that affect the tone of vascular smooth muscle and result in neutrophil adhesion and/or other autocrine and/or paracrine actions. NO, prostacyclin, prostaglandin E2 (PGE2), carbon monoxide (CO), tissue plasminogen activator, vascular endothelial growth factor (VEGF) and bFGF are endothelial mediators that reduce platelet and leukocyte activation, prevent thrombus formation, promote thrombolysis, maintain tissue perfusion, and protect the microvascular wall against acute damage[33,36-38,43-46]. For example, our previous study demonstrated that 16,16 dimethyl PGE2 protects human gastric mucosa against injury by 40% ethanol by protecting and preserving integrity of endothelial cells of...
gastric microvessels[47]. In response to wounding, infections or injurious stimuli, attachment between ECs is lost, resulting in increased endothelial permeability and edema[48].

The endothelium and blood vessels are integral parts of any tissue injury including COVID-19. Our previous studies demonstrated that ECs are critical targets of gastric mucosal injury by NSAIDs and ethanol, they initiate angiogenesis, and that age-related endothelial dysfunction of human and rat gastric endothelial cells results in impaired angiogenesis and delayed healing[19,20,24]. Our studies on aging gastropathy showed aging-related defects in ECs functions - angiogenesis, cell migration, proliferation, and healing of injury[19-21,49]. In a recent study, we also showed the critical role of mitochondria in aging gastric ECs; aging ECs have fewer mitochondria, and reduced mitochondrial membrane potential[50] that result in reduced ATP generation (Figure 2). We also demonstrated that treatment with VEGF and nerve growth factor (NGF) restores angiogenesis in cultured aging gastric ECs [20], accelerates healing of gastric ulcers and improves the quality of mucosal regeneration in vivo in aging rats[20,24].

### Endothelial cells and COVID-19

SARS-CoV-2 is a single, positive-stranded RNA virus that uses a spike-protein (S-protein) expressed on its envelope to bind to the host cell's human protein receptor ACE2[51-55]. The human ACE2 protein was initially identified as ACE-related carboxypeptidase membrane-associated and secreted enzyme expressed predominantly on the endothelium of the human heart, kidney, and testis [34]. However, it is widely expressed in various cells and tissues[55]. SARS-CoV-2 employs the ACE2 receptor, transmembrane serine protease 2 (TMPRSS-2), and cathepsin B and L (CTSB, and CTSL) for infection[51-53,56,57]. SARS-CoV-2 was detected in the respiratory tract, kidneys, liver, heart, and brain (all of which are highly vascularized tissues) of infected individuals[55]. ECs, which line the blood vessels of all organs and maintain microvascular integrity, express the ACE2 receptor and the cellular proteases TMPRSS-2, CTSB, and CTSL[57]. ECs are, therefore, a target for SARS-CoV-2 and blood vessels likely route of this virus dissemination to various organs. Electron microscopy (EM) and histologic studies detected SARS-CoV-2 virus-like particles and proteins in ECs of the kidney, small bowel, lung, myocardium, skin, and brain[35,13-16]. Ackerman et al.[15] showed abnormalities within the pulmonary microvasculature with congestion and micro-thrombi in lungs of COVID-19 patients, and visualized endothelial injury and lumen filled with cell fragments and degenerated organelles by electron microscopy. That study also showed increased ACE2-positive ECs and significant changes in endothelial morphology in lung autopsies of COVID-19 patients [15]. Varga et al.[5] using EM evaluation reported evidence of viral particles in renal ECs of COVID-19 patients presenting with endotheliitis, which is an immune and inflammatory response within the endothelium of blood vessels.

Other studies visualized SARS-CoV-2 proteins in dermal and renal endothelium[13,58]. While some studies were not able to corroborate presence of SARS-CoV-2 in ECs of some tissues, there is strong evidence to support that SARS-CoV-2 infects ECs. Monteil et al.[59] demonstrated that SARS-CoV-2 infects blood vessel organoids. SARS-CoV-2 virus particles range from approximate 70 to 120 nm[60-63]; therefore, in the absence of preexisting tissue injury, the virus would need to pass through the ECs to infect other tissues.

### Endothelial dysfunction

The term endothelial dysfunction was originally used to identify the shift from a normal quiescent endothelium to an impaired endothelium with the inability to generate nitric oxide and other vasodilators. In a broader definition, endothelial dysfunction includes impairment of endothelial function (that we used for aging endothelium in our previous papers) - reduced angiogenesis, pro-inflammatory, pro-vasoconstriction, proliferative, and pro-coagulant phenotype[18,64-66]. In certain pathological conditions characterized by preexisting endothelial dysfunction, the ACE/Ang II axis is upregulated resulting in vasoconstriction, thrombosis, fibrosis, coagulopathy, and thrombophilia.

### Endothelial dysfunction and endotheliitis in COVID-19

Emerging evidence indicates that preexisting endothelial dysfunction predisposes to COVID-19 infection and that COVID-19 induced endotheliitis further impairs endothelial integrity and function[27-30,32,34,67-76]. This is evidenced by the critical role of vascular endothelium in inflammation that results in dysregulation of cytokines...
Aging gastric endothelial cells (GECs) have significantly reduced mtMP reflecting impaired mitochondrial function vs young GECs [20], which is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/). GECs: Gastric endothelial cells.

The sequential steps of SARS-CoV-2 infection of ECs that result in endothelial pathology and a procoagulant, hypofibrinolytic state of the endothelium are summarized in Figure 3. SARS-CoV-2 utilizes the ACE2 receptors and cellular proteases (TMPRSS-2, CTSB and CTSL) infect the host cells including ECs [51-53,56,57]. The virus then replicates within the cells and is released into the blood vessels, which then disseminate the virus to distant organs. Severe COVID-19 results in increased production of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) which is referred to as cytokine storm [29,77,78]. The binding of IL-6 to its receptors on ECs increases vascular permeability, induces capillary leakage, and unleashes a cytokine storm by further increasing the secretion of IL-6, IL-8, and MCP-1 by ECs [29,70,78]. The cytokine storm in COVID-19 patients exposes the endothelium to pro-inflammatory cytokines resulting in leukocyte recruitment and inflammation and can lead to EC death that contributes to increased vascular permeability and end-organ damage [18,29]. In addition, activated ECs produce increased amounts of vWF and factor VIII, which participate in clot formation thereby inducing a pro-coagulant state. Furthermore, ECs produce increased amounts of PAI-1 that inhibits the degradation of clots and induces a hypofibrinolytic state [29,70,78].

The initial SARS-CoV-2 infection and vascular damage in pulmonary tissues can result in the release of ECs into the circulation. Increased numbers of circulating ECs (CECs) have been demonstrated in conditions associated with vascular damage [79-82]. Increased CECs may potentiate the spread to distant extrapulmonary tissues. Numerous extrapulmonary manifestations of SARS-CoV-2 infection such as acute kidney injury, thrombotic complications, myocardial dysfunction and arrhythmia, heart failure, venous thromboembolism, GI symptoms, hepatocellular injury, neurologic illnesses, ocular symptoms, and dermatologic complications have been documented [1]. Endothelial injury may be the underlying mechanism for both pulmonary and extrapulmonary manifestations of COVID-19.

**Endothelial cells are critical for vascular regeneration through angiogenesis and vasculogenesis during the injury/lesions healing phase**

The process of tissue injury healing involves tissue and vascular regeneration [32,34,75,83,84]. The latter is mediated by the sprouting of ECs from pre-existing vessels from areas bordering injury (angiogenesis), or the formation of new blood vessels from bone marrow-derived angiogenic precursor cells (vasculogenesis) [22,23,85]. Blood vessel reconstruction is regulated by angiogenic growth factors and involves the activation of genes such as basic fibroblast growth factor (bFGF or FGF-2) and its receptors; VEGF and its receptor; angiopoietins - Ang 1 and Ang 2, and their receptor, COX-2, serum response factor, NGF, stromal-derived factor II [25]. Our previous studies demonstrated the aging-related decrease in the expression of VEGF and NGF in ECs and that treatment with VEGF and NGF restore angiogenesis in aging gastric ECs (Figure 4) [20,21]. Furthermore, we showed that local NGF therapy of gastric ulcers increased in acute respiratory distress syndrome as well as multiple cardiovascular pathologies [18,27,30,64,71,73]. The ubiquitous expression of ACE-2 on ECs in all tissues suggests that SARS-CoV-2 can spread via circulation throughout the body and affect multiple organs [55].

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Figure 3 Sequential steps of SARS-CoV-2 infection of endothelial cells and endothelial damage. SARS-CoV-2 infects endothelial cells (ECs) using the host angiotensin-converting enzyme 2 receptors and cellular proteases (transmembrane serine protease 2, and cathepsin B and L). The virus then replicates within the cells and is released into the blood vessels, which then disseminate the virus to distant organs. Severe COVID-19 results in a cytokine storm wherein there is increased production of pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-1, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), and results in endothelial damage and endotheliitis, and demonstrated increased vascular permeability that cause plasma extravasation. Activated ECs produce increased amounts of vWF and factor VIII, and PAI-1, which induce a pro-coagulant, hypofibrinolytic state. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; ECs: Endothelial cells; ACE2: Angiotensin-converting enzyme 2; TMPRSS-2: Transmembrane serine protease 2; CTSB: Cathepsin B; CTSL: Cathepsin L; IL: Interleukin; TNF-α: Tumor necrosis factor-α.

The long-term effects of COVID-19 and its vaccines on endothelium and vasculature remains unknown

SARS-CoV-2 infection was first reported in 2019 and rapid, breakthrough research resulted in the development of several effective COVID-19 vaccines. Although these vaccines have proven effective in reducing the infection and severity of COVID-19, the long-term effects of the disease and the vaccines on ECs and blood vasculature are still to be determined.

POTENTIAL TREATMENTS

Two recent outstanding studies published by the Baishideng Publishing Group in the World Journal of Virology outlined the current therapies that have been utilized in COVID-19 treatment[86,87]. We wish to add to this list additional investigational treatments in ongoing clinical trials (Table 3) and describe two additional oral drugs that were announced in early November 2021 as potential COVID-19 treatments Molnupiravir (Merck) and Paxlovid (PF-07321332).

During recent press releases two newest oral drugs inhibiting SARS-CoV-2 replication were recently presented. Are they game changers? On November 4 and 5, 2021 two oral drugs were announced as novel COVID-19 treatments - Molnupiravir (Merck) and Paxlovid (PF-07321332). Both these drugs showed dramatic efficacy in controlling severe COVID-19 infection. The oral drug Molnupiravir (EIDD-2801) was developed by US-based Merck & Co Inc and Ridgeback Biotherapeutics[88] and investigated in a clinical trial (NCT04405570) to eliminate SARS-CoV-2 virus load in infected patients, has since been approved in the UK to treat patients with mild to moderate COVID-19 and at least one risk factor such as older age, diabetes, obesity, and heart disease that predisposes them for developing severe illness. Molnupiravir is the prodrug of the ribonucleoside analog β-D-N4-hydroxycytidine and is rapidly converted by host kinases in plasma to the active 5'-triphosphate form. The latter is a competitive substrate for SARS-CoV-2 RNA-dependent RNA polymerase and causes mutations in the viral genome during replication that makes the virus non-viable.

angiogenesis, promoted revascularization, and accelerated gastric ulcer healing in aging rats[20].
### Table 3 Summary of the Investigational Interventions/Treatments for COVID-19 in Clinical Trials

| Intervention/Treatment | Mode of action | Dose | Route | ClinicalTrials.gov Identifier |
|------------------------|----------------|------|-------|--------------------------------|
| Ronapreve/REGN-COV2 (REGN10933 and REGN10987) | Monoclonal antibodies against spike proteins | 8 g once, or 4 g twice | IV | NCT04425629 |
| Lopinavir/Ritonavir | Inhibitor of the HIV protease and cytochrome P-450 CYP3A | 200/ 50 mg; (4 tablets twice a day on day 1 followed by 2 tablets twice a day for 9 d) | Oral | NCT04403100 |
| Remdesivir (RDV, GS-5734, Veklury) | Inhibitor of RNA-dependent RNA polymerase | 200 mg on day 1 followed by 100 mg for 4-9 d | IV | NCT04292899 |
| Hyperimmune Plasma (COV19-PLASMA) | Immunotherapy | 250-300 mL up to 3 times over 5 d | IV | NCT04321421 |
| Tocilizumab (TCZ, ROACTEMRA) | Humanized anti-IL6 receptor monoclonal antibody | 8 mg/kg single infusion, up to 800 mg | IV | NCT04320615 |
| Sarilumab (Kevzara, REGN88, SAR1319) | Monoclonal antibody against IL-6 receptor alpha | 200 mg or 400 mg; single dose and multiple doses | IV | NCT04315298 |
| Anakinra (KINERET) | Monoclonal antibody against the IL-1 receptor | 100 mg daily up to 28 d | SC | NCT04330638 |
| Siltuximab (SYLVANT) | Chimeric anti-IL-6 antibody | 11 mg/kg single infusion | IV | NCT04330638 |
| Eculizumab | Monoclonal antibody against complement protein C3 | 900 mg every 7 d | IV | NCT04288713 |
| Methyl-prednisolone (MP) | Immunosuppression against cytokine storm | 80 mg/kg IV bolus, followed by infusion of 80 mg/d for at least 8 d and then oral MP 16 mg or 20 mg IV twice daily | Oral-IV | NCT04323592 |
| Heparin | Antithrombotic agents | 10 units/kg/h | IV | NCT04367831 |
| Enoxaparin (Lovenox) | Antithrombotic agents | 1 mg/kg | SC | NCT04367831 |
| Dexamethasone | Immunosuppression against cytokine storm | 20 mg/d (5 d) then 10 mg/d (5 d) | IV | NCT04325061 |
| Vitamin C | Antioxidant | 12 g infusion twice a day for 7 d | IV | NCT04264533 |
| Melatonin | Antioxidant | 3 or 30 mg three times a day for 14 d | Oral | NCT04784754 |
| CoQ10 | Antioxidant | 500 mg/day for 6 wk | Oral | NCT0490215 |

IL: Interleukin.

Specific action of this drug on SARS-CoV-2 infection of ECs is not known.

The second drug, Paxlovid (PF-07321332; ritonavir) is a SARS-CoV-2 protease inhibitor antiviral therapy[26]. PF-07321332 is an inhibitor of the SARS-CoV-2 3-chymotrypsin-like cysteine protease that is essential for SARS-CoV-2 replication[26,89]. Ritonavir is a protease inhibitor that slows down the metabolism/breakdown and therefore, increasing the bioavailability of other protease inhibitors including PF-07321332 in the body[90]. Studies published on November 2, 2021, in Science reported the discovery and characterization of PF-07321332 (Paxlovid)[26]. These studies demonstrated that Paxlovid inhibits SARS-CoV-2 replication in vitro in human adenocarcinoma-derived alveolar basal epithelial and differentiated normal human bronchial epithelial cells[26]. This drug showed in vitro coronavirus antiviral activity against all coronaviruses infecting humans and excellent off-target selectivity and in vivo safety profiles.

That study also showed the efficacy of orally administered 300 or 1000 mg/kg PF-07321332 against SARS-CoV-2 infection in vivo in a mouse model challenged intranasally with SARS-CoV-2 MA10 (CCID50). PF-07321332 Limited cellular infiltration by SARS-CoV-2 and protected lung tissue from damage compared to placebo treatment in that study[26]. Most importantly, the interim analysis of the Paxlovid human clinical trial demonstrated a dramatic approximate 90% reduction in COVID-19-related hospitalization or death in high-risk patients treated within 3 to 5 d of symptom onset compared to placebo. Since this drug inhibits virus replication the chance of endothelial infection and dissemination of virus via blood vessel is reduced. We postulate that ECs and blood vessels are likely an important part of this drug’s clinical efficacy. Naturally, this contention requires further careful analysis and confirmation, and in-depth insight, since the biological effects of these drugs are
Figure 4 Nerve growth factor gene therapy increases nerve growth factor expression and reverses impaired \textit{in vitro} angiogenesis in aging gastric endothelial cells. A: Nerve growth factor (NGF) gene therapy of aging Gastric endothelial cells (GECs) using lentiviral-NGF (LV-NGF) induced NGF expression (brown staining) and extensive, long filopodia (arrows) reflecting a change in these cells to an angiogenic phenotype; aging GECs without gene therapy (negative controls) have minimal NGF expression and lack filopodia; B: NGF gene therapy with LV-NGF resulted in 3.7-fold increased \textit{in vitro} angiogenesis at 6 h in aging GECs vs negative controls (control). Panels are representative images of capillary-like tube formation. Original magnification: \texttimes{} 200. Data are means \pm SD (\(n = 6\)). (**P < 0.001). NGF: Nerve growth factor; GEC: Gastric endothelial cells; LV: Lentiviral. Reproduced with permission from reference [20], which is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

CONCLUSION

While respiratory epithelium is the main route of virus entry, the ECs lining blood vessels are an integral part of COVID-19 disease progression and multi-organ spread. COVID-19 not only affects the lungs and respiratory system but also gastrointestinal tract, liver, pancreas, kidneys, heart, brain, and skin. Blood vessels serve as conduits for the virus dissemination to these distant organs. Importantly, ECs are also critical for vascular regeneration during injury/lesions healing and restoration of vascular network. In the present article, we reviewed the role of the endothelial and vascular components as major targets for COVID-19-induced tissue injury, spreading to various organs, and injury healing, and the current treatments for COVID-19 including the most recent oral drugs Molnupiravir and Paxlovid.

largely unknown[91,92]. This sentiment and discussion regarding these oral drugs are summarized in the November 10, 2021 Nature article titled COVID antiviral pills: what scientists still want to know[91]. On December 22, 2021, the US Food and Drug Administration issued an emergency use authorization of Paxlovid to treat mild and moderate COVID-19 (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-oral-antiviral-treatment-covid-19).

Other drugs that may be repurposed for COVID-19 treatment include melatonin, coenzyme Q 10 (CoQ10). Melatonin with its anti-inflammatory and anti-oxidative effects can protect against bacterial and viral infections[93-95] and an ongoing clinical study is investigating the efficacy of melatonin in COVID-19 (NCT: 04784754). A clinical trial is investigating the effect of high-dose CoQ10 in long-term COVID-19 patients (NCT: 04960215). The use of growth factors - VEGF, NGF, EGF and KGF, and treatment with adipose-derived stem cells (ADSCs) may be useful for COVID-19 therapy in both the initial and especially the regenerative, healing phase of the disease. A recent study demonstrated that ADSCs release exosomes that secrete various growth factors such as NGF, IGF1, HGF, etc.) that may alleviate the cytokine storm in COVID-19 patients[96].
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REFERENCES

1 Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, Bikdeli B, Akuwudike N, Ausiello JC, Wan EY, Freedberg DE, Kirtane AJ, Parikh SA, Maurer MS, Nordvig AS, Accili D, Bathon JM, Mohan S, Bauer KA, Leon MB, Krumholz HM, Uriel N, Mehra MR, Elkind MSV, Stone GW, Schwartz A, Ho DD, Bilezikian JP, Landry DW. Extrapulmonary manifestations of COVID-19. *Nat Med* 2020; 26: 1017-1032 [PMID: 32651579 DOI: 10.1038/s41591-020-09698-3]

2 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

3 Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J, Sordillo EM, Fowkes M. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Med Virol* 2020; 92: 699-702 [PMID: 32314810 DOI: 10.1002/jmv.25915]

4 South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. *Am J Physiol Heart Circ Physiol* 2020; 318: H1084-H1090 [PMID: 32228252 DOI: 10.1152/ajpheart.00217.2020]

5 Varga Z, Flammary AJ, Steiger P, Haberecker M, Andermatt Z, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H. Endothelial cell infection and endothelitis in COVID-19. *Lancet* 2020; 395: 1417-1418 [PMID: 32325026 DOI: 10.1016/S0140-6736(20)30937-5]

6 Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. *Nat Rev Gastroenterol Hepatol* 2021; 18: 269-283 [PMID: 33589829 DOI: 10.1038/s41575-021-00416-6]

7 Jiao L, Li H, Xu J, Yang M, Ma C, Li J, Zhao S, Wang H, Yang Y, Yu W, Wang J, Yang J, Long H, Gao J, Ding K, Wu D, Kuang D, Zhao Y, Liu J, Lu S, Liu H, Peng X. The Gastrointestinal Tract Is an Alternative Route for SARS-CoV-2 Infection in a Nonhuman Primate Model. *Gastroenterology* 2021; 160: 1647-1661 [PMID: 33370304 DOI: 10.1053/j.gastro.2020.12.001]

8 Sica R, Penmoni S, Penza L, Riccioni S, Di Cara G, Verrotti A. Gastrointestinal and hepatic involvement during COVID-19 pandemic: A focus on pediatric population and possible future implications. *World J Gastroenterol* 2021; 27: 7000-7004 [PMID: 34790020 DOI: 10.3748/wjg.v27.i40.7000]

9 Mohamed DZ, Ghoneim ME, Abu-Risha SE, Abdelsalam RA, Farag MA. Gastrointestinal and hepatic diseases during the COVID-19 pandemic: Manifestations, mechanisms, and management. *World J Gastroenterol* 2021; 27: 4504-4535 [PMID: 34366621 DOI: 10.3748/wjg.v27.i28.4504]

10 Wolff D, Nee S, Hickey NS, Marschollek M. Risk factors for Covid-19 severity and fatality: a structured literature review. *Infection* 2021; 49: 15-28 [PMID: 32860214 DOI: 10.1007/s00151-020-01509-1]

11 Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, Li Q, Jiang C, Zhou Y, Liu S, Ye C, Zhang P, Xing Y, Guo H, Tang W. Risk factors of critical & mortal COVID-19 cases: A systematic literature review. *JAMA Intern Med* 2020; 180: 934-943 [PMID: 32167524 DOI: 10.1001/jama.2020.0994]

12 Colmenero I, Santonja C, Alonso-Riaño M, Noguera-Morel L, Hernández-Martín A, Andina D, Wiesner T, Rodríguez-Peralto JL, Medina-Miguelañez M, Puebla L, Román-Curto C, Roncero-Riesco M, García R, Ortiz PL, Rodríguez-Peralto JL. Spectrum of Clinico-pathological Findings in COVID-19-induced Skin Lesions: Demonstration of Direct Viral Infection of the Endothelial Cells. *Am J Surg Pathol* 2021; 45: 293-303 [PMID: 3399338 DOI: 10.1097/PAS.0000000000001634]

13 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzanakou A, Li WW, Li VW, Mentzer SJ, Jonigk D. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med* 2020; 383: 120-128 [PMID: 32437596 DOI: 10.1056/NEJMoa2015432]

14 Bradley BT, Maioli H, Johnston R, Chaudhry I, Fink SL, Xu H, Najafian B, Deutsch G, Lacy JM, Williams T, Yarid N, Marshall DA. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet* 2020; 396: 320-332 [PMID: 32682491 DOI: 10.1016/S0140-6736(20)31305-2]
Tarnawski AS et al. Endothelium and blood vessels - targets for COVID-19

17 Samanta P, Ghosh AR. Environmental perspectives of COVID-19 outbreaks: A review. World J Gastroenterol 2021; 27: 5822-5830 [PMID: 34629805 DOI: 10.3748/wjg.v27.i35.5822]

18 Teuwen LA, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. Nat Rev Immunol 2020; 20: 389-391 [PMID: 32439870 DOI: 10.1038/s41577-020-0343-z]

19 Ahluwalia A, Jones MK, Hoa N, Tarnawski AS. NGF protects endothelial cells from indomethacin-induced injury through activation of mitochondria and upregulation of IGF-1. Cell Signal 2017; 40: 22-29 [PMID: 28843696 DOI: 10.1016/j.cellsig.2017.08.006]

20 Ahluwalia A, Jones MK, Hoa N, Zhu E, Brozowski T, Tarnawski AS. Reduced NGF in Gastric Endothelial Cells Is One of the Main Causes of Impaired Angiogenesis in Aging Gastric Mucosa. Cell Mol Gastroenterol Hepatol 2018; 6: 199-213 [PMID: 29992182 DOI: 10.1016/j.jcmgh.2018.05.003]

21 Ahluwalia A, Jones MK, Szabo S, Tarnawski AS. Aging impairs transcriptional regulation of vascular endothelial growth factor in human microvascular endothelial cells: implications for angiogenesis and cell survival. J Physiol Pharmacol 2014; 65: 209-215 [PMID: 24781730]

22 Risus W. Mechanisms of angiogenesis. Nature 1997; 386: 671-674 [PMID: 9109485 DOI: 10.1038/386671a0]

23 Risus W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol 1995; 11: 73-91 [PMID: 8689573 DOI: 10.1146/annurev.cb.11.110195.000445]

24 Jones MK, Kawanaka H, Baatar D, Szabó IL, Tsugawa K, Pai R, Koh GY, Kim I, Sarfath IH, Tarnawski AS. Gene therapy for gastric ulcers with single local injection of naked DNA encoding VEGF and angiopoietin-1. Gastroenterology 2001; 121: 1040-1047 [PMID: 11677194 DOI: 10.1053/gast.2001.29308]

25 Tarnawski AS, Ahluwalia A. The Critical Role of Growth Factors in Gastric Ulcer Healing: The Cellular and Molecular Mechanisms and Potential Clinical Implications. Cells 2021; 10 [PMID: 34440732 DOI: 10.3390/cells10081964]

26 Owen DR, Allerton CMN, Anderson AS, Aschenbrenner L, Avery M, Berritt S, Boras B, Cardin AD, Carlo A, Coffman KJ, Dantonio AD, Di L, Eng H, Ferre R, Gajiwala KS, Gibson SA, Greasley SE, Hurst BL, Kadar EP, Kalikutkar AS, Lee JC, Lee J, Liu W, Mason SW, Nodd S, Novak JJ, Obash RS, Ogilvie K, Patel NC, Pettersson M, Rai DK, Reese MK, Sammons MF, Sathish JG, Singh RSP, Steppan CM, Stewart AE, Tuttle JB, Updyke L, Verhoest PR, Wei L, Yang Q, Zhu Y. An oral SARS-CoV-2 M* inhibitor clinical candidate for the treatment of COVID-19. Science 2021; eabd4784 [PMID: 34726479 DOI: 10.1126/science.abd4784]

27 Nagashima S, Mendes MC, Camargo Martins AP, Borges NH, Godoy TM, Migliorano AFDRS, da Silva Deziderio F, Machado-Souza C, de Noronha L. Endothelial Dysfunction and Thrombosis in Patients With COVID-19-Brief Report. Arterioscler Thromb Vasc Biol 2020; 40: 2404-2407 [PMID: 32762443 DOI: 10.1161/ATVBAHA.120.314860]

28 Okada H, Yoshida S, Haru A, Ogura S, Tomita H. Vascular endothelial injury exacerbates coronavirus disease 2019: The role of endothelial glyocalyx protection. Microcirculation 2021; 28: e12654 [PMID: 32791568 DOI: 10.1111/micc.12654]

29 Pons S, Fodil S, Azoulay E, Zafrani L. The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. Crit Care 2020; 24: 353 [PMID: 32546188 DOI: 10.1186/s13054-020-03062-7]

30 Wazny V, Siau A, Wu KX, Cheung C. Vascular underpinning of COVID-19. Open Biol 2020; 10: 200028 [PMID: 32847471 DOI: 10.1098/rsob.200028]

31 Roumenina LT, Rayes J, Frimat M, Fremeaux-Bacchi V. Endothelial cells: source, barrier, and target of defensive mediators. Immunol Rev 2016; 274: 307-329 [PMID: 27782324 DOI: 10.1111/imr.12479]

32 Evans PC, Rainger GE, Mason JC, Guczki TJ, Osto E, Stamatakis Z, Neil D, Hoefer IE, Fragiadaki M, Woltenberger J, Weber C, Bochaton-Piallat ML, Bäck M. Endothelial dysfunction in COVID-19: a position paper of the ESC Working Group for Atherosclerosis and Vascular Biology, and the ESC Council of Basic Cardiovascular Science. Cardiovasc Res 2020; 116: 2177-2184 [PMID: 32750108 DOI: 10.1093/cvr/cvaa230]

33 Gryglewski RJ. Pharmacology of vascular endothelium. Delivered on 27 June 2004 at the 29th FEBS Congress in Warsaw. FEBS J 2005; 272: 2956-2967 [PMID: 15950556 DOI: 10.1111/j.1742-4658.2005.04725.x]

34 Alexander Y, Ostro E, Schmidt-Trucksäss A, Sheikh M, Trifunovic D, Daecker DJ, Aboyans V, Bäck M, Badimon L, Cosentino F, De Carlo M, Dorobantu M, Harousou DG, Guczki TJ, Hoefer IE, Morris PD, Norata GD, Saudes R, Taddei S, Vilahur G, Woltenberger J, Weber C, Wilkinson F, Bochaton-Piallat ML, Evans PC. Endothelial function in cardiovascular medicine: a consensus paper of the European Society of Cardiology Working Groups on Atherosclerosis and Vascular Biology, Aorta and Peripheral Vascular Diseases, Coronary Pathophysiology and Microcirculation, and Thrombosis. Cardiovasc Res 2021; 117: 29-42 [PMID: 32282914 DOI: 10.1093/cvr/cvaa085]

35 Deanfield J, Donald A, Ferri C, Giannattasio C, Halligan S, Lerman A, Mancia G, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Schiffrin EL, Taddei S, Webb DJ; Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. J Hypertens 2005; 23: 7-17 [PMID: 15643116 DOI: 10.1097/00004872-200501000-00004]

36 Michaels C. Endothelial cell functions. J Cell Physiol 2003; 196: 430-443 [PMID: 12891700 DOI: ]
Tarnawski AS et al. Endothelium and blood vessels-targets for COVID-19

10.1002/jcp.16333

37 Pries AR, Kuebler WM. Normal endothelium. *Handb Exp Pharmacol* 2006; 1-40 [PMID: 16999215 DOI: 10.1007/3-540-32967-6_1]

38 Sumpio BE, Riley JT, Dardik A. Cells in focus: endothelial cell. *Int J Biochem Cell Biol* 2002; 34: 1508-1512 [PMID: 1237270 DOI: 10.1016/s1357-2725(02)00075-4]

39 Bonfanti R, Furie BC, Furie B, Wagner DD. PADGEM (GMP-140) is a component of Weibel-Palade bodies of human endothelial cells. *Blood.* 1989; 73: 1109-1112 [PMID: 2467701]

40 McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest* 1989; 84: 92-99 [PMID: 2472243 DOI: 10.1172/JCI114175]

41 Mozijes A, Brehm MA. The Manifold Cellular Functions of von Willebrand Factor. *Cells* 2021; 10 [PMID: 34572000 DOI: 10.3390/cells10092351]

42 van Mourik JA, Romani de Wit T, Voorberg J. Biogenesis and exocytosis of Weibel-Palade bodies. *Histocomp Biol Cell Biol* 2002; 117: 113-122 [PMID: 11935287 DOI: 10.1007/s00418-001-0368-9]

43 Durante W. Carbon monoxide and bile pigments: surprising mediators of vascular function. *Vasc Med* 2002; 7: 195-202 [PMID: 12553743 DOI: 10.1111/j.1537-2995.2002.tb00246a]

44 Hanman RL, Kouroubas S, Flanders KC, Rogelj SJ, Roberts AB, Faller DV, Klagsbrun M. Endothelial cells synthesize basic fibroblast growth factor and transforming growth factor beta. *Growth Factors* 1988; 1: 7-17 [PMID: 32728801 DOI: 10.3109/097794889809000242]

45 Wu S, Wu X, Zhu W, Cai WJ, Schaper J, Schaper W. Immunohistochemical study of the growth factors, aFGF, bFGF, PDGF-AB, VEGF-A and its receptor (Flk-1) during angiogenesis. *Mol Cell Biochem* 2010; 343: 223-229 [PMID: 20559689 DOI: 10.1007/s11010-010-0517-3]

46 Ferrara N. VEGF-A: a critical regulator of blood vessel growth. *Eur Cytokine Netw* 2009; 20: 158-163 [PMID: 20167554 DOI: 10.1684/ecn.2009.0170]

47 Tarnawski A, Stachura J, Holander D, Sarfije B, Bogdal J. Cellular aspects of alcohol-induced injury and prostaglandin protection of the human gastric mucosa. Focus on the mucosal microvessels. *J Clin Gastroenterol* 1989; 10 Suppl 1: S35-S45 [PMID: 3183341 DOI: 10.1097/00004836-198812001-00008]

48 Shashby DM, Ries DR, Shasby SS, Winter MC. Histamine stimulates phosphorylation of adenosine diphosphate receptor junction proteins and alters their link to vimentin. *Am J Physiol Lung Cell Mol Physiol* 2002; 282: L1330-L1338 [PMID: 12003790 DOI: 11.1152/ajplung.00329.2001]

49 Tarnawski AS, Ahluwalia A, Jones MK. Increased susceptibility of aging gastric mucosa to injury: the mechanisms and clinical implications. *World J Gastroenterol* 2014; 20: 4467-4482 [PMID: 24782600 DOI: 10.3748/wjg.v10.i20.4467]

50 Ahluwalia A, Patel K, Hoa N, Brzozowska I, Jones MK, Tarnawski AS. Melatonin ameliorates aging-related impaired angiogenesis in gastric endothelial cells via local actions on mitochondria and VEGF-survivin signaling. *Am J Physiol Gastrointest Liver Physiol* 2021; 321: G682-G689 [PMID: 34668398 DOI: 11.1152/ajpgi.00101.2021]

51 Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY, Wang Q, Zhou H, Yan J, Qi J. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* 2020; 181: 894-904.e9 [PMID: 32278585 DOI: 10.1016/j.cell.2020.03.045]

52 Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intlensive Care Med* 2020; 46: 586-590 [PMID: 32124555 DOI: 10.1007/s00134-020-05955-9]

53 Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si JR, Zhu Y, Li B, Huang CL, Chen HD, Cao Y, Gao H, Jiang RD, Liu MQ, Chen Y, Shen XM, Zheng XS, Zhao K, Chen QJ, DENG F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579: 270-273 [PMID: 32015507 DOI: 10.1038/s41467-020-1227-7]

54 Donoghue M, Hsieh F, Barones E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woloff B, Robison K, Jeyaseelan R, Brebbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000; 87: E1-E9 [PMID: 10969042 DOI: 11.1161/01.res.87.5.e1]

55 Hikmet F, Mear L, Edvinsson Å, Mickle P, Uhlen M, Lindskog C. The protein expression profile of ACE2 in human tissues. *Mol Syst Biol* 2020; 16: e9610 [PMID: 32715618 DOI: 10.15252/msb.20209610]

56 Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Frichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; 181: 271-280.e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]

57 Sardu C, Gambardella I, Morelli MB, Wang X, Marfella R, Santulli G. Hypertension, Thrombosis, Kidney Failure, and Diabetes: Is COVID-19 an Endothelial Disease? *J Clin Med* 2020; 9 [PMID: 32403217 DOI: 10.3390/jcm9051417]

58 Puelles VG, Lütgehernmann M, Lindemeyer MT, Sperhake JP, Wong MN, Allweiss L, Chilla S, Heinemann A, Wanner N, Liu S, Braun F, Lu S, Pfefferle S, Schröder AS, Edler C, Gross O, Glatzel M, Wichmann D, Wiech T, Kluge S, Pueschel K, Aepfelbacher M, Huber TB. Multigorgan and Renal Tropism of SARS-CoV-2. *N Engl J Med* 2020; 383: 590-592 [PMID: 32402155 DOI: 10.1056/NEJMoa2011400]

59 Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, Leopoldi A, Garreta E, Hurtado
Del Pozo C, Prosper F, Romero JP, Wirsingberger G, Zhang H, Slutsky AS, Conder R, Montserrat N, Mirazimi A, Penninger JM. Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell* 2020; **181**: 905-913.e7. [PMID: 32333836 DOI: 10.1016/j.cell.2020.04.004]

Kim JM, Chung YS, Jo HJ, Lee NJ, Kim MS, Woo SH, Park S, Kim JW, Kim HM, Han MG. Identification of Coronavirus Isolated from a Patient in Korea with COVID-19. *Osong Public Health Res Perspect* 2020; **11**: 3-7. [PMID: 32149036 DOI: 10.24171/j.phrp.2020.11.1.02]

Park WB, Kwon NJ, Choi SI, Kang CK, Choe PG, Kim JY, Yun J, Lee GW, Seong MW, Kim NJ, Seo JS, Oh MD. Virus Isolation from the First Patient with SARS-CoV-2 in Korea. *J Korean Med Sci* 2020; **35**: e84. [PMID: 32080990 DOI: 10.3346/jkms.2020.35.e84]

Laue M, Kauter A, Hoffmann T, Möller L, Michel J, Nitsche A. Morphometry of SARS-CoV and SARS-CoV-2 particles in ultra thin plastic sections of infected Vero cell cultures. *Sci Rep* 2021; **11**: 3515. [PMID: 33568700 DOI: 10.1038/s41598-021-82852-7]

Liu C, Mendonça L, Yang Y, Gao Y, Shen C, Liu J, Ni T, Ju B, Liu C, Tang X, Wei J, Ma X, Zhu Y, Liu W, Xu S, Liu Y, Yuan J, Wu J, Liu Z, Zhang Z, Liu L, Wang P, Zhang P. The Architecture of Inactivated SARS-CoV-2 with Postfusion Spikes Revealed by Cryo-EM and Cryo-ET. *Structure* 2020; **28**: 1218-1224.e4. [PMID: 33058760 DOI: 10.1016/j.str.2020.10.001]

Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003; **23**: 168-175. [PMID: 12588755 DOI: 10.1161/01.ath.0000051384.43104.fc]

Sena CM, Pereira AM, Seiça R. Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta* 2013; **1832**: 2216-2231. [PMID: 23994612 DOI: 10.1016/j.bbadia.2013.08.009]

Fauci AS, Lane HC, Redfield RR. Covid-19 - Navigating the Uncharted. *N Engl J Med* 2020; **382**: 1268-1269. [PMID: 32109011 DOI: 10.1056/NEJMc2002387]

Froldi G, Kaur S. The COVID-19 Cytokine Storm; What are the age influences. *Med Hypotheses* 2020; **144**: 110015. [PMID: 32592919 DOI: 10.1016/j.mehy.2020.11.015]

Gavriilaki E, Anfanti P, Gavriilaki M, Lazaridis A, Douma S, Gkaliagkousi E. Endothelial Dysfunction in COVID-19: Lessons Learned from Coronavirus. *Curr Hypertens Rep* 2020; **22**: 63. [PMID: 32852642 DOI: 10.1007/s11906-020-01078-6]

Hayden MR. Endothelial activation and dysfunction in metabolic syndrome, type 2 diabetes and coronavirus disease 2019. *J Int Med Res* 2020; **48**: 300060520939746. [PMID: 32722979 DOI: 10.1177/0306056020939746]

Kaur S, Tripathi DM, Yadav A. The Enigma of Endothelium in COVID-19. *Front Physiol* 2020; **11**: 989. [PMID: 32848893 DOI: 10.3389/fphys.2020.00989]

Mosich W, Chen K, Pfau SE, Vashist A. Endotheliitis and Endothelial Dysfunction in Patients with COVID-19. Its Role in Thrombosis and Adverse Outcomes. *J Clin Med* 2020; **9**: 32549229. [DOI: 10.3390/jcm9061862]

Huertas A, Montani D, Savale L, Pichard C, Hubert F, Guignabert C, Humbert M. Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? *Eur Respir J* 2020; **56**: 32554538. [DOI: 10.1183/13993003.01634-2020]

Amraei R, Rahimi N. COVID-19, Renin-Angiotensin System and Endothelial Dysfunction. *Cells* 2020; **9**: 32660065. [DOI: 10.3390/cells9071652]

Jung F, Krüger-Genge A, Franke RP, Hufert F, Küpper JH. COVID-19 and the endothelium. *Clin Hemorheol Microcirc* 2020; **75**: 7-11. [PMID: 32568187 DOI: 10.3339/ch.20-200907]

Karakaş M, Jarczak D, Becker M, Roedl K, Addo MM, Hein F, Bergmann A, Simon K, Kwon NJ, Choi SJ, Kang CK, Choe PG, Kim JW, pharmacodynamic study of adenosine A1 receptor agonists in humans. *J Interv Cardiol* 2020; **33**: 1182-1189. [PMID: 32732251 DOI: 10.3390/fmmu.2021.1295193]

Ragag D, Salah Eldin H, Taeimah M, Khatib R, Saleem R. The COVID-19 Cytokine Storm; What We Know So Far. *Front Immunol* 2020; **11**: 1446. [PMID: 32612617 DOI: 10.3389/fimmu.2020.01446]

Farinaceli M, Krahn T, Dinh W, Volk HD, Düngen HD, Wagner J, Konen T, van Ahsen O, van Ahsen O, van Ahsen O. Circulating endothelial cells as biomarker for cardiovascular diseases. *Res Pract Thromb Haemost* 2019; **3**: 49-58. [PMID: 30656276 DOI: 10.1002/rth2.12158]

Abdel Hamid M, Bakhoum SW, Sharaf Y, Sabry D, El-Gengehe AT, Abdel-Latif A. Circulating Endothelial Cells and Endothelial Function Predict Major Adverse Cardiac Events and Early Adverse Left Ventricular Remodeling in Patients With ST-Segment Elevation Myocardial Infarction. *J Interv Cardiol* 2016; **29**: 89-98. [PMID: 26864952 DOI: 10.1111/jicc.12269]

Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical
relevance. *Circulation* 2007; 115: 1285-1295 [PMID: 17353456 DOI: 10.1161/CIRCULATIONAHA.106.652859]

82 Hill JM, Zalis G, Halcox JP, Schenke WH, Wacławiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; 348: 593-600 [PMID: 12584367 DOI: 10.1056/NEJMoa022287]

83 Gambardella J, Khondkar W, Morelli MB, Wang X, Santulli G, Tramuraco V. Arginine and Endothelial Function. *Biomedicines* 2020; 8 [PMID: 32781795 DOI: 10.3390/biomedicines8080277]

84 Cotran R, Kumar V, Robbins S, editors. Robbins Pathologic Basis of Disease. 5th Edition ed. Philadelphia: Saunder, 1999; 298-299, 773-777

85 Ahluwalia A, Brzozowski T, Jones MK, Ichikawa Y, Tarnawski AS. Formation of new blood vessels during gastric ulcer healing. Role of bone marrow derived endothelial progenitor cells. *J Physiol Pharmacol* 2017; 68: 585-589 [PMID: 29151075]

86 Di Franco S, Alfieri A, Petrou S, Damiani G, Passavanti MB, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; 348: 593-600 [PMID: 12584367 DOI: 10.1056/NEJMoa022287]

87 Iturricastillo G, Ávalos Pérez-Urría E, Couñago F, Landete P. Scientific evidence in the COVID-19 treatment: A comprehensive review. *World J Virol* 2021; 10: 217-228 [PMID: 34631473 DOI: 10.5501/wjv.v10.i5.217]

88 Fischer W, Eron JJ, Holman W, Cohen MS, Fang L, Szewczyk LJ, Sheahan TP, Baric R, Mollan KR, Wolfe CR, Duke ER, Azizad MM, Borroto-Esoda K, Wohl DA, Loftis AJ, Alabanza P, Lipansky F, Painter WP. Molnupiravir, an Oral Antiviral Treatment for COVID-19. *medRxiv* 2021 [PMID: 34159342 DOI: 10.1101/2021.06.17.21258639]

89 Lea AP, Faulds D. Ritonavir. *Drugs* 1996; 52: 541-6; discussion 547 [PMID: 8891466 DOI: 10.2165/00003495-199652040-00007]

90 Ledford H. COVID antiviral pills: what scientists still want to know. *Nature* 2021; 599: 358-359 [PMID: 34759341 DOI: 10.1038/d41586-021-03074-5]

91 Zhou S, Hill CS, Sarkar S, Tse LV, Woodburn BMD, Schinazi RF, Sheahan TP, Baric RS, Heise MT, Swanstrom R, β-d-N4-hydroxycytidine Inhibits SARS-CoV-2 infection. *Curr Opin Virol* 2021; 49: 36-40 [PMID: 34029993 DOI: 10.1016/j.coovi.2021.04.006]

92 Mazini L, Rochette L, Malka G. Exosomes contribution in COVID-19 patients' treatment. *J Transl Med* 2021; 19: 234 [PMID: 34059065 DOI: 10.1186/s12967-021-02884-5]
