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Review Article

The Role of Smoking and Nicotine in the Transmission and Pathogenesis of COVID-19

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Running Title: Smoking could worsen COVID-19 outcome

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Abbreviations

COVID-19: Coronavirus disease of 2019

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

WHO: World Health Organization

ACE2: Angiotensin converting enzyme 2

RAS: Renin angiotensin system

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CoVs: Coronaviruses

MERS: Middle East respiratory syndrome

COPD: Chronic obstructive pulmonary disease

Ro: Basic reproductive number

CDC: Centers for disease control and prevention

ICU: Intensive care unit

HEV 67N: Hemagglutinating encephalomyelitis

e-Cig: Electronic cigarette

FDA: US Food & Drug Administration

BBB: Blood-brain barrier

CNS: Central nervous system

GBS: Guillain-Barré syndrome

AT_1R: Angiotensin type-1 receptor

CVPD: Cardiovascular and pulmonary diseases

nAChR: Nicotinic acetylcholine receptor

IQOS: I quit original smoking

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Abstract

The coronavirus disease of 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, is turning out to be one of the most devastating global pandemics in the history of humankind. There is a shortage of effective therapeutic strategies or preventative vaccines for this disease to date. A rigorous investigation is needed for identifying and developing more effective therapeutic strategies for COVID-19. Angiotensin converting enzyme 2 (ACE2), a crucial factor in COVID-19 pathogenesis, has been identified as a potential target for COVID-19 treatment. Smoking and vaping are potential risk factors for COVID-19 which are also shown to upregulate ACE2 expression. In this review, we have discussed the pathobiology of COVID-19 in the lungs and brain and the role of ACE2 in the transmission and pathobiology of this disease. Further, we have shown possible interactions between nicotine/smoking and ACE2 in the lungs and brain which could aggravate the transmission and pathobiology of COVID-19 resulting in a poor disease outcome.

Significance Statement

This review addresses the present global pandemic COVID-19 with respect to its pathobiology in the lungs and brain. It focuses on the potential negative impact of tobacco and nicotine exposure on the outcomes of this disease by interaction with the ACE2 receptor. It adds to the time-sensitive and critically important growing knowledge about the risk factors, transmission, pathobiology, and prognosis of COVID-19.

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Introduction

Coronavirus disease of 2019 (COVID-19) has become the most significant health hazard in the world since the flu pandemic in 1918. It is the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. Approximately 27.5 million people worldwide have been infected with the virus which has resulted in 894,983 deaths as of September 9, 2020 (World Health Organization, 2020b). The virus emerged from the city of Wuhan, Hubei Province, China in December 2019 (Li et al., 2020b). Due to its significantly high contagiousness (Du et al., 2020; Li et al., 2020b; Riou and Althaus, 2020), the virus quickly spread throughout the world. On March 11, 2020, the World Health Organization (WHO) declared the outbreak as a pandemic. More than 6.3 million people and counting in the US have been infected with SARS-CoV-2 and 189,147 deaths from COVID-19 have been reported as of September 9, 2020 (Centers for Disease Control and Prevention, 2020a). The transmission and pathogenesis of the SARS-CoV-2 is shown to be, at least in part, mediated by angiotensin converting enzyme-2 (ACE2) receptor which is a crucial part of the renin angiotensin system (RAS).

Coronaviruses (CoVs) have been the major causes for the recent outbreaks of respiratory disease around the world. The name “Coronaviruses” came along because of the viruses’ shape which resembles that of a crown, as corona is the Latin for crown. CoVs are large enveloped RNA viruses with a single strand which can be isolated from a number of animal species (Perlman and Netland, 2009). These viruses can transmit into different species and cause several illnesses which could range from the common cold to severe diseases like Middle East respiratory syndrome (MERS) and SARS. Seven human-infecting CoVs have been identified till date. Large type 1 transmembrane spike (S) glycoproteins give the CoVs their distinguishing

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crown-like feature. This cell surface S proteins are heavily glycosylated and contain two specific functional domains. These domains (S1 and S2) are hypothesized to facilitate virus' entry into the host cell. The S1 domain contains the ACE2 receptor-binding domain and is responsible for the initial entry into the host cell (Li et al., 2005). The S2 domain helps the virus in the fusion with the cell membrane that is essential for cellular penetration (Coutard et al., 2020). Enzymatically modified S proteins expose the fusion site for cellular adhesion. Protein convertases called furin, highly expressed in the lungs, mediate this process through cleavage by cellular proteases (Follis et al., 2006; Coutard et al., 2020). The SARS-CoV-2 has a diameter of approximately 60-140 nm with shapes ranging from round to elliptic while it can also be pleomorphic. The genome of this virus contains 29891 nucleotides which encode for 9860 amino acids. It has a significant level of genetic similarity with the SARS-like-CoVZXC21 found in bats and the SARS-CoV found in humans (Chan et al., 2020). Hence the name SARS-CoV-2 was designated. The source of the virus could be linked with a distinct bat strain and a number of possible intermediate hosts could also exist for this virus including pangolins (Zhang et al., 2020). Initially, it was thought that SARS-CoV-2 is mostly transmitted from animal to human, but later it was found that human-to-human transmission is another possible transmission route for this virus. It is mostly spread by symptomatic people, although the asymptomatic spread is also possible (Qian et al., 2020). It is suggested that close contact between individuals is needed for SARS-CoV-2 spread. The transmission can occur through respiratory droplets from coughing and sneezing. Aerosol transmission is also possible in case of extended exposure to increased aerosol concentrations in closed spaces. The unique aspect of this virus is its contagiousness, expressed by its basic reproductive number (Ro) which is estimated to be between 2.2 to 2.7 (Du et al., 2020; Li et al., 2020b; Riou and Althaus, 2020) and it has an incubation period of 2-14

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days. The mortality rate of COVID-19 was estimated to be 3.4% by WHO (World Health Organization, 2020a). Currently, no vaccine or therapeutic drug is available for SARS-CoV-2 except for remdesivir and convalescent plasma which are approved for emergency use by the US Food and Drug Administration (FDA).

Smoking is one of the most important risk factors for aggravated COVID-19 transmission and outcome. It remains one of the primary causes of preventable diseases and death in the US (2014). Although the smoking rate around the world is on the decline, there has been a recent rise of electronic cigarette (e-Cig), an alternative tobacco product, partly because of its perceived safety and widespread popularity. The short and long-term health effects of these nicotine-containing devices are still unknown and warrant extensive research. Since tobacco smoke and chronic vaping can cause chronic obstructive pulmonary disease (COPD), it can make people more susceptible to serious lung complications of COVID-19. Tobacco smoking and e-Cigs can also weaken lungs and the immune system, putting a person at increased risk for serious complications with COVID-19 (Bhatta and Glantz, 2020). Currently, the centers for disease control and prevention (CDC) consider people with chronic lung disease or moderate to severe asthma as a high risk for severe illness from COVID-19. A major clinical question exists related to if the CDC should place smokers and/or vapers in the high-risk category for being seriously impacted by the SARS-CoV-2, even if they do not have COPD. Although clinical data comprising all epidemiological factors are still scarce due to the ongoing progression of the pandemic, preliminary studies show a possible link between smoking and higher mortality rate in COVID-19 (Kaur et al., 2020; Vardavas and Nikitara, 2020). The act of smoking itself makes smokers likely to be more vulnerable to this disease by increasing the possibility of transmission of the virus from hand to mouth (World Health Organization, 2020c). In a systematic review

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based on five clinical studies, Vardavas and Nikitara calculated that smokers were 1.4 times more likely to have severe symptoms of COVID-19 and approximately 2.4 times more likely to be admitted to an intensive care unit (ICU), requiring mechanical ventilation or die (Vardavas and Nikitara, 2020). Also, COVID-19-associated hospitalization rates were higher in males than females (5.1 versus 4.1 per 100,000 population) during March 1-28, 2020 in the US (Garg, 2020). This data can be potentially linked with smoking and COVID-19 mortality as males are more likely to be current cigarette smokers (Centers for Disease Control and Prevention, 2020b). Smokers have been demonstrated to be at a higher risk to contract influenza with aggravated symptoms. They were also shown to have increased mortality in the previous MERS-CoV outbreak (Arcavi and Benowitz, 2004; Park et al., 2018). Recent reports have shown that nicotine and tobacco smoke exposure can upregulate ACE2 expression in different organs of the body. In this article, we explore the role of ACE2 in COVID-19 disease outcome and how smoking can modulate this receptor in lung and brain, two of the most vital body organs, potentially resulting in enhanced SARS-CoV-2 infection and worsened COVID-19 prognosis & mortality rate.

Pathobiology of COVID-19 in the Lungs and Brain

The complete pathobiology of COVID-19 has not been fully elucidated. The lungs are the principal organs affected by the virus due to its airborne transmission route. Other organs in the body, e.g. heart & brain, also show signs and symptoms of direct or indirect viral injury. Cardiopulmonary symptoms are seen in the critical COVID-19 cases and can progress to acute respiratory distress syndrome, organ failure and sepsis in severe cases due to an overactivation of the immune system termed as ‘cytokine storm’ (Guan et al., 2020a). Here, we have primarily focused on the pathological effects of the SARS-CoV-2 on the lungs and brain.

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Lung

The severity of the SARS-CoV-2 infection depends on the region of the lung affected. Mild symptoms involve the upper respiratory airways whereas the severe stages of the infection occur in the lower respiratory tract. COVID-19 can be divided into three phases that correspond to different clinical stages of the disease (Mason, 2020; Wu and McGoogan, 2020). The asymptomatic stage lasts for the initial 1-2 days of infection where the inhaled virus attaches to the nasal epithelial cells. Individuals become infectious at this point despite having a low viral titer. The second stage involves the upper respiratory airways. The virus replicates and moves down the respiratory tract which creates a stronger and robust immune response. The symptoms of COVID-19 disease is observable at this stage. Approximately, 20% of the infected patients advance to the third stage of COVID-19 disease which is a severe condition characterized by pulmonary infiltrates. At this stage, the virus reaches the lower respiratory tract, the site for gaseous exchange, and induces a number of pathological changes which include bilateral diffuse alveolar damage, hyaline membrane formation, pulmonary edema, interstitial mononuclear inflammatory infiltrates, multinucleated syncytial cells, and amphophilic granular cytoplasm (Xu et al., 2020). Alveolar type II cells are preferentially affected over Type I cells in both influenza and SARS-CoV (Mossel et al., 2008; Weinheimer et al., 2012). In a recently published study, human alveolar type II cells infected with SARS-CoV showed release of viral particles leading to cellular apoptosis and the resultant decrease of Type II cells also led to secondary epithelial regeneration (Qian et al., 2013). Kumar et al reported similar results in a murine model of influenza pneumonia (Kumar et al., 2011). Fever and dry cough are some of the most common symptoms (Chen et al., 2020a; Huang et al., 2020a) which are seen at the initial stages of COVID-19. However, the most typical COVID-19 symptom is respiratory distress. Dyspnea is

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the marker for a severe form of the disease which is a dangerous condition requiring immediate medical attention. Among the patients experiencing dyspnea, more than half need ICU treatment. Conditions of most of the ICU patients deteriorated quickly resulting in death due to respiratory failure (Li et al., 2020d). Current research will help fill the pathogenesis gaps between the COVID-19 damage to lungs to further improve pulmonary function for these patients and to test potential anti-viral agents.

Brain

The SARS-CoV-2 infection has been shown to exert different neurotropic actions (Archie and Cucullo, 2020). It has been demonstrated that β-CoVs to which the SARS-CoV-2 belongs, also affect the central nervous system (CNS) besides the respiratory tract (Steardo et al., 2020). This property was seen in the SARS-CoV, MERS-CoV, and the coronavirus responsible for porcine hemagglutinating encephalomyelitis (HEV 67N). Because of the resemblance between SARS-CoV and SARS-CoV-2, it is highly probable that SARS-CoV-2 could also hold a similar potential. SARS-CoV-2 can breach the blood-brain barrier (BBB) during the course of ongoing infection. Based on an epidemiological survey, the median time from the first symptom of COVID-19 to dyspnea, hospital admission, and ICU were 5, 7, and 8 days respectively (Wang et al., 2020a). This latent period gives the virus enough time to enter the nervous system and infect the brain of COVID-19 patients. BBB disruption is more likely to happen in severe cases of the infection and can be associated with adverse immunological reactions such as the cytokine storm (Channappanavar and Perlman, 2017; Mao et al., 2020b). The virus can remain latent in the neural cells but it can also produce harmful neurologic symptoms affecting vascular homeostasis, adaptive immunity, and cognitive function. Neuroinvasion is hypothesized to be a potential mechanism for the pathophysiology and clinical manifestations of COVID-19 (Steardo et al.,

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2020) which could also play a vital role in the respiratory failure of the patients (Li et al., 2020d). A recent retrospective report suggests that over 30% of 814 COVID-19 patients showed neurologic symptoms (Mao et al., 2020b). Loss of sense of smell (anosmia) frequently occurs in COVID-19 patients (Gane et al., 2020). Furthermore, several studies have reported other neurologic signs such as nausea, vomiting, and headache (Li et al., 2020d). Guan and colleagues found less frequent neurologic symptoms such as headache (13.6%) and myalgias (14.9%) in a group of 1099 SARS-CoV-2 infected patients (Guan et al., 2020b), but neurologic involvement carried a poor prognosis for COVID-19 patients. In a concurrent study, Mao et al. found that about 88% of the severe COVID-19 patients showed neurologic symptoms which include acute cerebrovascular disorders and defective consciousness (Mao et al., 2020a). There were also reports of large vessel stroke in the SARS-CoV outbreak which broke out in Singapore in 2004 (Umapathi et al., 2004). Coagulopathy and vascular endothelial dysfunction have also been suggested as potential complications of COVID-19 (Zhou et al., 2020a). In light of that, there have been recent reports of unexplainable acute large vessel strokes in young COVID-19 patients (Oxley et al., 2020). Five cases of large vessel stroke in COVID-19 patients under the age of 50 were reported over 2 weeks. The stroke rate was found to be approximately 5% in a retrospective study with hospitalized patients in Wuhan, China (Li et al., 2020c). The youngest patient in that study was 55 years old. Similarly, Beyrouti et al. suggested that an elevated systemic prothrombotic state could play a role in COVID-19-linked ischemic stroke incidents (Beyrouti et al., 2020).

COVID-19 may affect the nervous system by different hypothesized mechanisms which could also overlap. It could directly affect the nervous tissue as seen with herpes simplex encephalitis. It has been hypothesized that SARS-CoV-2 can enter the nervous system via the

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ACE2 receptor, which is present in glial cells and neurons. Another possible mechanism to invade the CNS include hematogenous spread and retrograde neuronal transmission via olfactory neurons in the cribriform plate (Ding et al., 2004; Gu et al., 2005a; Xu et al., 2005; Netland et al., 2008). Research with the similar SARS-CoV indicate that these viruses can also directly enter the brain. Direct neuroinvasion of SARS-CoV-2 into the medullary cardiorespiratory center can contribute to the respiratory failures seen in COVID-19 patients (Ding et al., 2004; Gu et al., 2005b; Xu et al., 2005; Netland et al., 2008; Li et al., 2020d). RNAs of SARS-CoV have been found, almost exclusively in neurons, in the cerebrospinal fluid and brain tissue of SARS-CoV-infected patients (Ding et al., 2004; Gu et al., 2005a; Xu et al., 2005), while brain penetration of SARS-CoV via the olfactory system can also happen as observed in mice (Netland et al., 2008). The brainstem was demonstrated to be heavily infected by the SARS-CoV (McCray et al., 2007; Netland et al., 2008). This evidence can be linked with the olfactory and taste disorders reported in some COVID-19 patients. The second type of brain injury can occur due to an aggravated immune response which has been described previously as a cytokine storm. Cytokines can cross the BBB and are linked with acute necrotizing encephalopathy. One such case linked with COVID-19 has been reported (Poyiadji et al., 2020). The third mechanism of CNS damage could result from untoward host immune response to an acute infection which can be exemplified by Guillain-Barré syndrome (GBS). One case of GBS associated with COVID-19 has been reported although with limited evidence for cause and effect (Zhao et al., 2020a). The fourth mechanism of indirect viral damage results from the effects of systemic illness. Neurologic symptoms like encephalopathy, critical illness myopathy, and neuropathy are commonly seen in COVID-19. Most cases of neurologic complications linked with the disease would appear to fall into this class.

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Role of ACE2 in the Transmission and Pathobiology of COVID-19

The renin-angiotensin system (RAS) is an important factor in the regulation of physiological parameters including blood pressure (BP) (Xia and Lazartigues, 2008). The RAS can follow two different pathways resulting in either tissue injury or protection. The physiological functions and interplay between different RAS components have been summarized in fig 1. ACE2 is a type I transmembrane metallocarboxypeptidase homologous to ACE. ACE2 was identified in 2000 (Donoghue et al., 2000; Tipnis et al., 2000) and was initially reported to be expressed in the heart, kidney and testis (Donoghue et al., 2000; Tipnis et al., 2000). Later studies showed a ubiquitous ACE2 expression all over the body (Igase et al., 2005; Sakima et al., 2005; Doobay et al., 2007). ACE2 metabolizes Ang-I to Ang-(1–9) and Ang-II to Ang-(1–7) (Ferrario, 2011). These metabolites exert potent vasodilator and other protective effects which are mediated by the Mas receptor which could also negatively regulate the RAS (Crackower et al., 2002; Ferrario, 2011). Our lab investigates the function and regulation of the brain endopeptidase neurolysin, which increases formation of Ang-(1-7) and other brain specific substrates, as a potential target to preserve the brain after stroke injury (Rashid et al., 2014; Jayaraman et al., 2020). The physiological role of ACE2 in human health is complex and has not been fully elucidated yet, but possible protective effects of ACE2 against lung injury (Imai et al., 2005) and oxidative stress (Xia et al., 2011) have been observed in experimental studies.

Both bioinformatics modeling and in vitro experiments indicate that SARS-CoV-2 likely utilizes ACE2 as a receptor to gain entry into human cells (Zhou et al., 2020b). ACE2 was also previously identified as an entry receptor for SARS-CoV and HCoV-NL63 (Li et al., 2007). ACE2 expression level may enhance SARS-CoV-2 binding and infectivity and this hypothesis warrants further investigation. SARS-CoV has been detected in brains of infected patients,

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almost exclusively in neurons, suggesting possible distribution of ACE2 to the CNS (Ding et al., 2004; Gu et al., 2005a; Xu et al., 2005). The expression of ACE2 is increased significantly 24 h after SARS-CoV infection and remains at a high level after 48 h (Li et al., 2020a). This indicates that ACE2 not only plays a critical role in viral susceptibility but may also be involved in post-infectious regulation. The high expression of ACE2 was related to enhanced inflammatory responses which could be linked with the symptoms of a cytokine storm. A clinical study in Wuhan also found that the levels of some pro-inflammatory markers such as IL-1β, IL-10 and IL-8 were significantly increased in critically ill patients with new COVID-19 infection (Huang et al., 2020a). This may be associated with pyroptosis, which has been suggested as another pathological mechanism involved in this infection. Pyroptosis is an inflammatory form of apoptosis. Li et al. found that high expression of ACE2 in lung tissue induced a cytotoxic reaction, neutrophil inflammation and a Th2-dominated immune response. Moreover, the expression of ACE2 varies in a time-dependent manner after SARS-CoV infection. Although ACE2 has been shown to exert anti-inflammatory effects in experimental studies (Rodrigues Prestes et al., 2017), Li et al. suggested that ACE2 expression could be linked with the activation of several inflammatory mediators including neutrophils, natural killer cells, T-helper cells, dendritic cells and TNF-α secreting cells. These events can result in an aggravated inflammatory reaction in SARS-CoV which could be an indirect effect of the virus binding to cell surface ACE2 (Li et al., 2020a).

Another potential role of ACE2 (Fig 1), seemingly paradoxical to its role in viral transmission and inflammation, in COVID-19 has been suggested in the literature (Guo et al., 2020; Li et al., 2020d). It was discovered that infection with SARS-CoV led to the downregulation of the ACE2 receptor (Glowacka et al., 2010). From a physiological standpoint,

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the ACE2 receptor serves as a negative regulator of severe lung edema and lung injury by decreasing Ang-II levels (Imai et al., 2005). Ang-II-induced vascular permeability and lung injury is facilitated by binding to the AT_1R (Jia, 2016). In a mouse model, SARS-CoV-induced lung damage was shown to be attenuated by AT_1R antagonism (Kuba et al., 2005b). If SARS-CoV (and possible SARS-CoV-2) binding to ACE2 causes downregulation of ACE2, this could further result in higher Ang-II levels and increased lung injury. Multiple ACE2 knockout mouse models have demonstrated the protective effects of ACE2 from lung injury and vascular inflammation (Gu et al., 2016). ACE2, being a significant negative regulator of RAS in the cardiovascular system plays a significant role in the control of BP, the higher mortality in Chinese COVID-19 patients with hypertension may be related to that phenomenon. Additionally, ACE2 null mice display reduced cardiac contractility. These results may explain why cardiac impairment is observed more frequently in patients who die from COVID-19 (Kuba et al., 2010; Thomas et al., 2010). Inhibitors of the ACE2 and RAS have been suggested as potential therapeutic strategies for COVID-19 (Vaduganathan et al., 2020). Contrastingly, it was also suggested that ACE inhibitor or AT_1 receptor blocker therapy may increase the risk and sensitivity to COVID-19 (Fang et al., 2020) but studies have not found any significant association between the use of these drugs and COVID-19 outcome and prognosis (Díaz-Guardiola et al., 2020; Huang et al., 2020b). The complex interplay of ACE2 and the RAS system on COVID-19 pathobiology has not been fully elucidated and additional mediators or receptors other than ACE2 could assist viral binding and host entry, which warrant further investigation. There is also a hypothesis that enhanced expression of soluble ACE2 may function as a competitive interceptor of SARS-CoV-2 which can slow cellular entry of virus, thereby mediating protective effects against lung injury (Batlle et al., 2020). Although no receptor other

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than ACE2 has been connected with the viral binding and entry, cellular proteases can play an important role in the cellular entry of SARS-CoV-2 by facilitating viral activation and engulfment. These include the transmembrane protein serine 2, TMPRSS2 (Leung et al., 2020), Cathepsin B/L (Sungnak et al., 2020), and serine protease inhibitors (serpins) (Dittmann et al., 2015) which need to be investigated for any potential benefits in COVID-19. Moreover, combination therapies targeting multiple mechanisms of the viral entry into the host cell may provide better therapeutic efficacy.

Smoking and Vaping: Possible Risk Factors for COVID-19

The severity of COVID-19 outcome can be affected by various risk factors and coexisting conditions. Age, cardiovascular diseases, chronic COPD, asthma, pulmonary fibrosis and interstitial lung disease, type 1 and type 2 diabetes are all shown to be potential risk factors for worsened COVID-19 outcomes (Leung et al., 2020)(Mason, 2020). Another potential risk factor which can affect the clinical outcome of COVID-19 is smoking, but this is not listed as a CDC risk factor. A review of studies by public health specialist which was convened by WHO on 29 April 2020 found that smokers are more vulnerable to develop severe COVID-19 disease compared to non-smokers (World Health Organization, 2020e). Another recent meta-analysis established an association between smoking and COVID-19 (Patanavanich and Glantz, 2020). Smoking is a crucial factor in determining a person's ability to develop and manage viral infection, especially a respiratory infection (Razani-Boroujerdi et al., 2004; Eddleston et al., 2011). Smokers have more respiratory diseases including colds than non-smokers. The influenza rate is twice in smokers and they have also enhanced rates of bacterial pneumonia and tuberculosis (Atto et al., 2019; Eapen et al., 2019).

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Cigarette smoking is the leading cause of premature death in the world (Jha, 2009). Smoking can also enhance the risks for other risk factors for COVID-19. It is also the single most important risk factor for the development of cardiovascular and pulmonary diseases (CVPD), and smokers are two to four times more likely to develop CVPD than nonsmokers (2014; Jamal et al., 2018). A causal relationship has been established between smoking and coronary heart disease, atherosclerotic aortic aneurysm, cerebrovascular disease, stroke, acute respiratory illnesses, COPD, and exacerbation of asthma (2014). Cigarette smoking has been in a steady decline since the 1950s, but the introduction of e-Cig 10 years ago has gained interest from former smokers as well as a new generation of consumers. Nicotine is a highly addictive substance, and it is currently unclear whether e-Cig use, commonly known as vaping, is "safer" than regular cigarette use or whether they can overturn the health gains, mostly on the cardiopulmonary system, achieved with the reduction of tobacco smoking. It is a matter of significant concern that nicotine inhalation devices are becoming increasingly popular in the young generation (Wagoner et al., 2016), which calls for greater awareness and more investigations into the potential cardiopulmonary risks of nicotine-containing products. Not enough attention has been given to the role of smoking in either the transmission of the novel SARS-CoV-2 or COVID-19 mortality rate (Brake et al., 2020). Smokers have higher susceptibility to COVID-19 as the act of smoking itself enhances the possibility of virus' transmission from hand to mouth (World Health Organization, 2020d). Further, smoking-induced lung injury enhances the susceptibility to viral and bacterial pulmonary infections (Lawrence et al., 2019). Preclinical research studies have associated e-Cig exposure with increased pulmonary inflammation, oxidative stress, inhibition of the immune system, mucus hypersecretion, and protease-mediated lung damage, which is a similar pathogenesis seen from

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exposure to combustible tobacco products (Moretto et al., 2012; Chun et al., 2017). A recent longitudinal analysis also showed that e-Cig use, like combustible tobacco smoking, is a separate risk factor for respiratory diseases (Flacco et al., 2020). Interestingly, dual use (E-cig and combustible tobacco products) was the most common usage trend and was found to be riskier than using a single product. WHO reported that a significant number of COVID-19-related deaths are linked with comorbid conditions (Zheng et al., 2020). Smoking can also negatively impact the severity of COVID-19 outcome by interaction with the RAS system. Table 1 lists retrospective observational studies which established a correlation between smoking and COVID-19 severity &/or mortality.

Effects of Smoking on COVID-19: Role of nAChRs and ACE2 Receptors

Nicotine and smoking exposure can modulate ACE2 expression in the lungs and other major organs of the body which suggest that smoking could promote COVID-19 cellular entry through nicotinic acetylcholine receptors (nAChRs) signaling. nAChRs are found in cells that also express ACE2 in the lungs, kidneys, heart, brain and other organs (Changeux, 2010; Tolu et al., 2013; Nordman et al., 2014). Smoking can thus affect the pathogenesis and prognosis of COVID-19 in many organs.

Lung

Nicotine inhaled from tobacco smoke or e-Cig first exerts its action on the lung. nAChRs are expressed in different lung cell types which include type II alveolar epithelial cells, alveolar macrophages, bronchial epithelial cells, interstitial fibroblasts, and pulmonary endothelial cells (Conti-Fine et al., 2000; Oakes et al., 2018b). These cell types also express the RAS components.

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Studies have provided suggestions that smoking and vaping may increase the risk of SARS-CoV-2 infections although these have not been directly proven yet (Lockett et al., 2012; Jackson et al., 2020; Smith and Sheltzer, 2020). Some contrasting studies have even shown a lower prevalence of smoking in COVID-19 patients (Farsalinos et al., 2020a; Tajlil et al., 2020). Researchers have also demonstrated that smokers may have a worsened outcome of COVID-19 after contracting the virus (Del Sole et al., 2020; Kaur et al., 2020). Nicotine can functionally and quantitatively enhance lung ACE. Li et al. found that ACE2 level was markedly upregulated in long-term smokers (Li et al., 2020a). Smokers and individuals with COPD are also shown to have an increased airway expression of ACE2. These results are consistent with previous studies in rodents which showed that smoke exposure upregulated both the expression and activity of airways' ACE2 (Yilin et al., 2015; Hung et al., 2016). While the upregulation of ACE2 may play a beneficial role in protecting the host against acute lung damage, with chronic exposure, this may create an enhanced risk of CoV infections in smokers. This would partially account for the enhanced susceptibility of active smokers to viral respiratory tract infection and also virus-induced complications in smokers who had COPD. Researchers, using an ex vivo lung perfusion model, showed that smoking exposure for one day increased the conversion of Ang-I to Ang-II in rats (Bakhle et al., 1979). In a recent study, Cai et al. also showed an enhanced pulmonary ACE2 gene expression in smokers (Cai et al., 2020). They also showed that furin, the protein convertase, was upregulated in the lungs by smoking. This study observed that smoking induces hyperplasia of goblet cells and a reduction of club cells in the bronchial epithelium. ACE2 gene was also shown to be primarily expressed in goblet cells in smokers and club cells in never-smokers. This result is consistent with another very recent study that found that ACE2 is mostly expressed in club cell-derived Alveolar Type II cells and in a transient secretory cell type in

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subsegmental bronchial branches (Lukassen et al., 2020). Brake et al. observed a higher ACE2 level in smokers' lungs with or without COPD (Brake et al., 2020). ACE2 was notably expressed in COPD patients which suggests that COPD can further exaggerate ACE2 and expose possible SARS-CoV-2 attachment sites. Results from previous studies showed that attachment of SARS-CoV-2 to ACE2 could in turn decrease ACE2 expression. Consequently, other relevant ACE enzymes were upregulated causing severe acute respiratory failure (Kuba et al., 2005a; Zhao et al., 2020b). Wang et al. also concluded that ACE2 could be linked with COVID-19 and smoking (Wang et al., 2020b). Another study demonstrated dose-dependent upregulation of ACE2 by smoking in rodent and human lungs (Smith and Sheltzer, 2020). They also showed that chronic smoking causes hyperplasia of goblet cells in the respiratory epithelium which was linked with enhanced ACE2 expression. Further, ACE2 is shown to be significantly expressed in type-2 pneumocytes that have a high expression of genes regulating viral reproduction and transmission (Brake et al., 2020; Zhao et al., 2020b). Researchers (Podowski et al., 2012) demonstrated that chronic cigarette smoke exposure with losartan treatments showed a protective action against the injurious effects exerted by smoking exposure which include enhanced oxidative stress in lung, emphysema, fibrosis, and apoptosis of alveolar septal cell in mice. In this study, cigarette smoke upregulated AT_1R expression in the lung parenchyma, which was reversed by losartan. The ratio of AT_1R to AT_2R increased five to six fold in bronchiole areas with enhanced fibrosis, which were associated with diminished lung function in COPD patients (Bullock et al., 2001). A recent study also showed that sub-chronic e-Cig exposure enhances lung inflammation and ACE2 expression which were shown to be mediated by α7 nAChRs (Wang et al., 2020c). Maremanda et al. have demonstrated transcriptional changes in genes regulating mitochondrial activity, cellular senescence, and telomere length might contribute to smoking related chronic lung

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disorders in association with COVID-19 infection (Maremanda et al., 2020). These results indicate that smokers and/or vapers may have an increased risk of COVID-19 related complications which can aggravate disease severity and treatment outcome.

It is important to note, that while smokers with significant lung disorders are more sensitive to worsened prognosis of COVID-19, evidence of similar worsening effects in healthy smokers has been scarce. Studies involving COVID-19 patients in China have also shown that smoking prevalence in COVID-19 patients was lower than the estimated expected prevalence (Farsalinos et al., 2020a). Confounding factors have to carefully considered while interpreting these findings including the absence of control groups and the descriptive nature of the studies. Also, inherent differences in demographic variables of the populations could make a comparison between the smoking prevalence in hospitalized COVID-19 patients and that of overall population estimates inappropriate (Reddy et al., 2020). Researchers have further suggested possible beneficial effects of nicotine in COVID-19 (Farsalinos et al., 2020b). These effects are thought to be mediated by the anti-inflammatory effects of nicotine (Ulloa, 2005; Mabley et al., 2011) by its interaction with the cholinergic nervous system, notably α7 nAChRs. Exploring the potential of nicotine as a therapeutic agent in the treatment of COVID-19 seems intriguing, however, with respect to smoking/vaping, these suggestions should be carefully examined since acute and chronic exposures and multiple doses of nicotine might alter the observed beneficial effects on the cholinergic nervous system. Moreover, the pro-inflammatory effects of other components of tobacco smoke/e-Cig could offset any beneficial effects exerted by nicotine. Contrary to this notion, a recent study by Wang et al. showed that nicotine containing e-Cig exposure can increase the level of inflammatory mediators in the lung which was mediated by α7

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nAChRs (Wang et al., 2020c). Further studies are definitely warranted to determine the effects of nicotine on inflammation.

Brain

Nicotine primarily exerts its brain effects by binding to neuronal nAChRs, which are omnipresent throughout the CNS and play a crucial role in neuronal excitation in autonomic ganglia (Dani and Bertrand, 2007). Nicotine could affect autonomic nuclei by interacting with the brain RAS (Xu et al., 2011). Furthermore, exposure to nicotine is shown to alter both the expression and the activity of the brain RAS system. Perinatal nicotine exposure is also demonstrated to alter the brain RAS resulting in enhanced sympathetic function in the adult offspring. Baroreflex sensitivity was decreased after Ang-II infusion in rats which were prenatally exposed to nicotine (Xiao et al., 2008; Yu et al., 2017). Perinatal nicotine exposure can also increase brain AT_1R and decrease brain AT_2R expression in offspring rats (Lu et al., 2008). ACE2, the target receptor of SARS-CoV-2, is found in the brain and shown to functionally modulate nAChRs (Ferrari et al., 2007; Oakes et al., 2018a). ACE2 signaling is hypothesized to act as a counteractive mechanism against oxidative stress and neuroinflammation. It also counteracts the impairment of the activity and stability of ACE that can lead to enhanced neurodegenerative loss of dopaminergic neurons (Labandeira-García et al., 2014). Impaired ACE activity could affect cholinergic pathways in the brain cortex and can thereby play a role in Alzheimer's disease' progression (Kehoe et al., 2009). Significant ACE2 mRNA expression was found in different regions of the adult human brain (Jones et al., 2009) which also express various nAChR subtypes (Dani and Bertrand, 2007). Our lab has further shown that long-term nicotine exposure can upregulate neuronal α7 nAChR in ischemic

condition (Sifat et al., 2018). These make smokers' brain cells more susceptible to the infection as nicotine can upregulate ACE2 expression of these cells through the nAChRs (Olds and Kabbani, 2020). This is a significant matter to consider as mRNA analysis of infected patients reveals that the similar SARS-CoV, which also uses ACE2 to enter the host cell, was present in the brain and cerebrospinal fluid (Zhang et al., 2003; Chong et al., 2004; Inoue et al., 2007). Additionally, the capacity of SARS-CoV to enter neurons has been shown previously (Netland et al., 2008; Kaparianos and Argyropoulou, 2011). Virtually every systemic RAS component is present in the brain (Xu et al., 2011; Oakes et al., 2018b). As Ang-II cannot easily enter the brain because of the BBB, the function of brain RAS is not dependent on the systemic RAS (Schelling et al., 1976). Endogenous Ang-II has been detected in different brain regions including hypothalamus and brainstem (Lind et al., 1985). Fig 2 outlines a possible mechanistic pathway through which nicotine can upregulate ACE2 receptor expression to facilitate the entry of SARS-CoV-2 in neurons resulting in different neurological complications. Although several studies involving smoking and lung ACE2 expression have been conducted, effects of smoking and vaping on brain ACE2 expression are scarce which warrants further investigations to explore the role of smoking in neurologic and cerebrovascular complications of COVID-19.

COVID-19 can cause unexplained and abnormal blood coagulation leading to large vessel stroke in young people as mentioned earlier. Tobacco smoke and/or e-Cig exposure can potentially worsen that complication. Our lab and others have previously shown that nicotine and tobacco smoke exposure can worsen ischemic stroke outcome (Paulson et al., 2006; Paulson et al., 2010; Kaisar et al., 2017; Matsuo et al., 2020). We have specifically shown that tobacco smoke or e-Cig exposure can downregulate circulating thrombomodulin level which suggests a pro-coagulant pre-disposition and increased risk of stroke (Kaisar et al., 2017). Nicotine and

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tobacco smoke exposure are also associated with aggravated brain inflammation & oxidative stress, and also reduced brain glucose utilization in ischemic stroke conditions (Kaisar et al., 2017; Sifat et al., 2018). On top of these, e-Cig is becoming increasingly popular among the young generation because of its perceived safety and lack of proper education (Murthy, 2017). Rigorous research is needed to elucidate the link between large-vessel stroke and young COVID-19 patients and how smoking &/or vaping can potentially modulate this interaction possibly through ACE2 receptors.

Conclusion

COVID-19 is the most recent global pandemic that has already caused over 894,983 deaths worldwide so far. Due to the fast human to human contagiousness of the SARS-CoV-2 virus, cases have easily spread from China to the whole world. People all over the world are being home quarantined and maintaining social distance to fight off the contraction of this virus. There is a dire need for preventive vaccines and therapeutic agents for battling this worldwide pandemic. FDA has approved emergency use authorizations for remdesivir and convalescent plasma for the treatment of COVID-19 in hospitalized patients. Several other potential therapeutics and vaccines are under investigation and development. This review explores the possible role of smoking and/or e-Cig usage in the transmission, pathobiology, and prognosis of the COVID-19 disease. We have focused on the mechanistic pathways involving nicotine and ACE2 receptors of the brain and lung RAS system, which appear to play a significant role in virus entry and COVID-19 pathobiology in humans. Research has shown that smoking and possibly e-Cig exposure can upregulate the ACE2 receptors in the brain and lung which make smokers more vulnerable to the COVID-19 disease. Coexisting conditions are identified as significant risk factors for COVID-19 mortality. This accentuates the link between smoking and

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COVID-19 vulnerability as smoking is already established to increase the risk for several comorbidities including diabetes, cardiovascular disease, and COPD (Rojewski et al., 2016). The association between smoking and the risk for enhanced SARS-CoV-2 infection should be investigated further with better study design adjusting for confounding factors. The mechanisms underlying smoking mediated upregulation of ACE2 pulmonary expression are not fully understood yet which warrants further investigations. Genetic polymorphisms of ACE2 should also be taken into consideration with respect to smoking/vaping-linked COVID-19 vulnerability, sensitivity, and clinical prognosis. Although the popularity of e-Cigs is on the rise, few studies have explored the effects of e-Cig exposure on lung and brain ACE2 expression. Further research involving possible injurious effects of smoking on COVID-19 outcomes should also include e-Cigs and other alternative smoking devices like waterpipes and "heat-not-burn" devices, such as 'I quit original smoking (IQOS)' devices. Further, as the SARS-CoV-2 has the potential to invade the brain and cause various neurologic and cerebrovascular complications, postmortem brains of COVID-19 patients, both smokers and nonsmokers, should be analyzed whenever possible. Continuous monitoring for evidence of viral injury beyond the lungs, heart, and kidney is also required. We also propose that rigorous research should be directed towards ACE2 as it has the potential to be a valuable therapeutic target for SARS-CoV-2. Especially, the interplay between ACE2 and nAChRs in the lungs and brain should be carefully investigated. Pathogenesis of large vessel ischemic stroke in smoking and nonsmoking COVID-19 patients is another crucial research area. Importantly, people should consider taking the necessary steps to reduce smoking and vaping rates as smoking can aggravate transmission, pathobiology, and disease outcomes of COVID-19.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Sifat, Nozohouri, Villalba, Vaidya, Abbruscato.

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Footnotes

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Table 1. A list of retrospective observational studies which provided a correlation between smoking and COVID-19 severity &/or mortality.

Article Title(s)	Study Setting	Sample Number	Positive Smoking Status, Number (Percentage)		Smoking And COVID-19 Severity, Number (Percentage)			Smoking And COVID-19 Mortality, Number (Percentage)
Associations of clinical characteristics and antiviral drugs with viral RNA clearance in patients with COVID-19 in Guangzhou, China: a retrospective cohort study (Chen et al., 2020b)	Guangzhou Eighth People's Hospital (Jan 20-Mar 15, 2020)	284	28 (9.9)		Asymptomatic	2 (7.1)	P= 0.838	
					Mild	3 (10.7)		
					Moderate	17 (60.7)		
					Serious	5 (17.9)		
					Critical	1 (3.6)		
Preliminary Estimates of the Prevalence of	CDC passive surveillance	74439				Hospitalized (Non-ICU)	ICU	
			Former		Former smoker	33 (7)	7 (1)	

Selected Underlying Health Conditions Among Patients with Coronavirus Disease 2019 — United States, February 12–March 28, 2020 (COVID et al., 2020)	registry (-March 28, 2020)		smoker	165 (2.3)				
			Current smoker	96 (1.3)	Current smoker	5 (1)	8 (2)	
The clinical characteristics of COVID-19: a retrospective analysis of 104 patients from the outbreak on board the Diamond Princess cruise ship in Japan (Tabata et al., 2020)	Passengers and crew of Diamond Princess cruise ship	104	18 (17.3)					
Risk Factors Associated with	Tianyou Hospital	323	38 (11.8)		Non-severe	12 (7.9)	P= 0.123	
					Severe	22 (15.1)		

Clinical Outcomes in 323 COVID-19 Patients in Wuhan, China (Hu et al., 2020)	(Jan 8- Feb 20, 2020)			Critical	4 (15.4)				
				Unfavorable outcome	12 (19)	P= 0.045			
				Favorable outcome	26 (10)				
Development and external validation of a prognostic multivariable model on admission for hospitalized patients with COVID-19 (Xie et al., 2020)	Tongji Hospital (Jan- Feb 2020)	299	10 (3.4)				Survival	5 (3.5)	P= 0.914
							Death	5 (3.2)	
Epidemiological and clinical features of 125 Hospitalized Patients with COVID-19 in Fuyang, Anhui, China	NO.2 People's Hospital of Fuyang City (Jan 20- Feb 9, 2020)	125	16 (12.8)	Non-critical	9 (9)	P= 0.027			
				Critical	7 (28)				

Epidemiological and clinical features of 125 Hospitalized Patients with COVID-19 in Fuyang, Anhui, China (Wang et al., 2020d)									
Prognostic value of C-reactive protein in patients with COVID-19 (Luo et al., 2020b)	Eastern Campus of Renmin Hospital of Wuhan University (Jan 30-Feb 20, 2024)	298	21 (7)				Recovered	13 (6.1)	P= 0.295
							Died	8 (9.5)	
Characteristics of patients with	Eastern Campus of	403	29 (7.2)				Recovered	20 (6.6)	P= 0.421

COVID-19 during epidemic ongoing outbreak in Wuhan, China (Luo et al., 2020a)	Renmin Hospital, Wuhan University (Jan 30-Feb 25, 2020)						Died 9 (9)	
Clinical course and risk factors for mortality of adult Inpatients with COVID-19 in Wuhan, China: a retrospective cohort study (Zhou et al., 2020a)	Jinyintan Hospital and Wuhan Pulmonary Hospital (Wuhan, China) who had been discharged or had died by Jan 31, 2020	191	11 (6)	OR 95% CI	2.23 (0.65, 7.63)	P =0.2		

Epidemiological and clinical features of 2019-nCoV acute respiratory disease cases in Chongqing municipality China: a retrospective descriptive multiple-center study (Qi et al., 2020)	Qianjiang central hospital of Chongqing, Chongqing three gorges central hospital and Chongqing public health medical center (Jan 19- Feb 16, 2020)	267	53 (19.9)		Non-severe	22 (10.1)	P<0.001	
					Severe	31 (62)		
Clinical Characteristics of Coronavirus Disease 2019 in China (Guan	552 hospitals in China (Dec 11, 2019-	1099	Former Smoker	21 (1.9)		Former Smoker	Current Smoker	
					Non-severe	12 (1.3)	108 (11.8)	

et al., 2020b)	Jan 29, 2020)		Current Smoker	137 (12.6)	Severe	9 (5.2)	29 (16.9)	

ICU- Intensive Care Unit, CDC- Center for Disease Control and Prevention, COVID-19- Coronavirus Disease 2019, OR- Odds Ratio, 95% CI- 95% Confidence Interval

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Figure Legends

Figure 1. Possible interactions between SARS-CoV-2 and the renin-angiotensin system: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to cell surface angiotensin converting enzyme 2 (ACE2) receptor with its spike protein for host cell entry. After endocytosis of the virus, surface ACE2 is downregulated but ACE remains unchanged. This event alters the balance of the renin-angiotensin system (RAS). Angiotensin II (Ang-II) is increased and the injurious effects of this hormone (vasoconstriction, inflammation, fibrosis etc.), mediated by angiotensin type 1 receptor (AT_1R), can result in excessive tissue damage, e.g. lung tissue damage. On the other hand, the concomitant decrease in Ang-(1-7) & Ang-(1-9) hinders the protective effects (anti-apoptotic, anti-inflammation, anti-fibrosis etc.) of these hormones, mediated by Mas receptor (MasR), causing further tissue injury.

Figure 2. Hypothetical mechanism of nicotine-induced facilitation of SARS-CoV-2 entry into neurons through ACE2 receptors: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can enter the central nervous system through different mechanism(s) (hematogenous spread, retrograde olfactory neuronal transmission, cytokine storm, and compromised blood-brain barrier) and bind to its proposed receptor, angiotensin converting enzyme 2 (ACE2), almost exclusively in neurons. The virus then enters the neurons through endocytosis and starts replication which could lead to a number of neurological complications. In the brain, nicotine acts by binding to neuronal nicotinic acetylcholine receptors (nAChRs) and also interacts with the brain renin angiotensin system (RAS). Nicotine exposure results in an upregulation of nAChRs which can subsequently increase cell surface ACE2 expression. Excessive ACE2 could competitively bind with SARS-CoV-2 and enhance the viral entry and replication in neurons. This enhanced viral binding to ACE2 could in turn downregulate the

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expression of ACE2 receptor causing an imbalance in the brain RAS. As a result, the detrimental effects of Ang-II, mediated by angiotensin type 1 receptor (AT_1R), would be increased while the protective effects of Ang-(1-7) & Ang-(1-9), mediated by Mas receptor (MasR), would be decreased. The overall effects of these mechanisms could result in an excessive neuronal infection and damage by SARS-CoV-2.

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Figure 1.

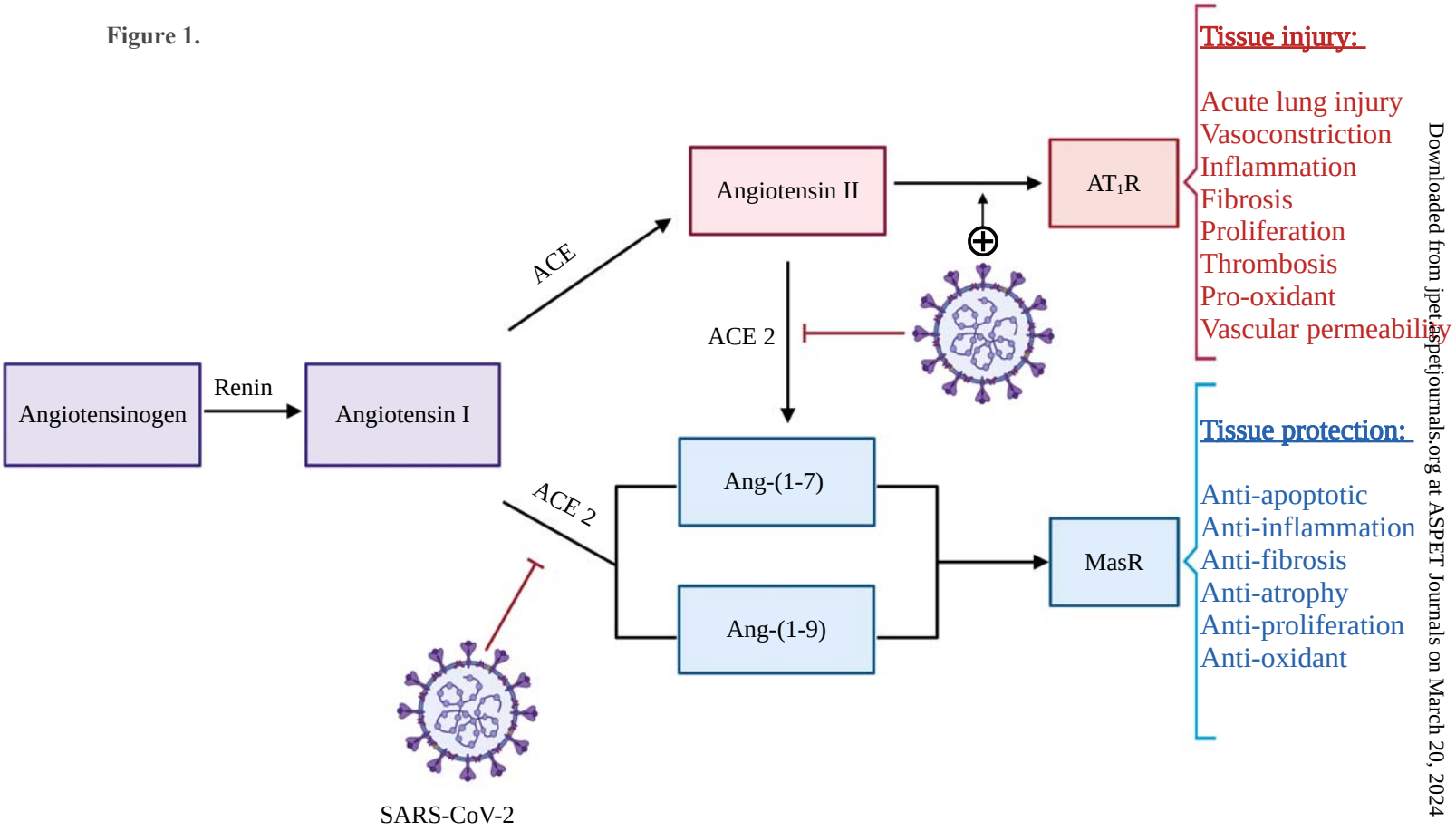

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Figure 2.