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ABSTRACT

The world is currently going through a serious pandemic of viral infection with SARS-CoV-2, a new isolate of coronavirus, resembling and surpassing the crisis that occurred in 2002 and 2013 with SARS and MERS, respectively. SARS-CoV-2 has currently infected more than 142,000 people, causing 5,000 deaths and reaching more than 130 countries worldwide. The very large spreading capacity of the virus clearly demonstrates the potential threat of respiratory viruses to human health, alarming governments around the world that preventive health policies and scientific research are pivotal to overcoming the crisis. Coronavirus disease 2019 (COVID-19) causes flu-like symptoms in most cases. However, approximately 15% of patients will need hospitalization, and 5% require assisted ventilation, depending on the cohorts studied. What is intriguing,
however, is the higher susceptibility of elderly individuals, especially those who are more than 60 years old and have comorbidities, including hypertension, diabetes and heart disease. In fact, the death rate in this group may be up to 10-12%. Interestingly, children are somehow protected and not included as a risk group.

Thus, here, we discuss some possibilities of molecular and cellular mechanisms by which elderly subjects may be more susceptible to severe COVID-19. In this sense, we raise two main points: i) increased ACE-2 expression in pulmonary and heart tissue of chronic angiotensin 1 receptor (AT1R) blocker users and hypertensive individuals and ii) antibody-dependent enhancement (ADE) after previous exposure to other circulating coronaviruses. We believe these are pivotal points for a better understanding of the pathogenesis of severe COVID-19 and must be addressed with attention by physicians and scientists in the field.

INTRODUCTION

The world is facing another major public health crisis with the pandemic spread of the recently described coronavirus named SARS-CoV-2\(^1\)\(^-\)\(^3\). Reaching proportions that greatly surpass that of SARS and MERS, the SARS-CoV-2 epidemic started in Wuhan, China, in December 2019 but has now reached more than 130 countries worldwide and infected approximately 142,000 people with more than 5,000 deaths (WHO, March 13\(^{th}\) 2020)\(^4\). Sequencing analysis of the viral genome has evidenced mutations in the spike protein, which is essential for viral attachment and invasion into host cells, that may have favored the spill over from bats to humans\(^1\). Although most patients infected with coronaviruses develop a mild flu-like disease, in which the most common symptoms are fever and cough, according to Guan W et al 2020\(^5\), studying 1,099 patients from 552
hospitals from 30 provinces of China, 15.7% of patients evolve to severe disease with increasing difficulties in breathing due to pneumonia. Radiological imaging of the lungs evidenced opacity in 56.4% of the patients. Approximately 2.7% of patients needed assisted ventilation, and only 1.4% died\(^1\).

Coronavirus disease 2019 (COVID-19), however, may rapidly evolve to severe acute respiratory syndrome (SARS) in elderly subjects (>60 yo), especially in those with comorbidities, as hypertension, diabetes and pulmonary diseases\(^{1,4,6}\). What is intriguing is that, unlike influenza virus\(^7\), children are not included in the risk group, as very few cases of severe COVID-19 have been reported, and there were no deaths of children under the age of 9. This raises questions on the cellular and molecular mechanisms associated with COVID-19 severity. Understanding and elucidating such mechanisms may greatly improve our knowledge of the pathogenesis of the disease and thus guide health professionals on how to better approach the elderly population.

In this sense, we propose to raise two main points of discussion: i) the increased angiotensin converting enzyme-2 (ACE-2) expression in pulmonary and heart tissues of hypertensive patients under chronic use of AT1R blockers and ii) antibody-dependent enhancement (ADE) after previous exposure to another circulating coronaviruses.

**SARS-CoV-2 and ACE-2**

After entering the host, usually through aerosolized viral particles or contact with contaminated surfaces, the virus needs to undergo its biological cycle. Spike proteins that are coded by the S gene on one of the open reading frames of the viral genome need to interact with viral receptors on the surface of host cells. Coronavirus spike proteins attach to angiotensin-converting enzyme-2 (ACE-2), which is mainly present in
epithelial cells of the lungs\textsuperscript{8,9}. This is the main reason why coronaviruses often cause respiratory disease. Notably, ACE-2 may also be highly expressed in intestinal tissues\textsuperscript{9}, leading to diarrhea, as observed in 60\% of patients during the SARS-CoV epidemic in 2002. Only in a few cases of SARS-CoV-2 had diarrhea, although viral particles may be detected in stool\textsuperscript{10}. After attaching to ACE-2 through the receptor-binding domain (RBD) of the S1 and S2 domains of the spike protein, the viral envelope fuses with the host cell membrane and is further internalized. Genetic material, a positive RNA strand of approximately 20-32 kb, is released into the cytoplasm for replication\textsuperscript{1}. Thus, the importance of ACE-2 dynamics of expression for viral infectivity, tropism and pathogenicity is evident.

ACE-2, or ACE-related carboxypeptidase, is an 805 amino acid transmembrane protein with a pivotal role in the regulation of blood pressure through the conversion of oligopeptides of the angiotensin family members in the so-called renin-angiotensin system\textsuperscript{11}. Whereas ACE converts angiotensin I (Ang I) to angiotensin II (Ang II), ACE-2 converts Ang II to angiotensin 1-7 (Ang 1-7) or angiotensin 1-9 (Ang 1-9)\textsuperscript{12}. Ang II and Ang 1-7 have antagonizing effects, as Ang II binds to angiotensin 1 receptor (AT1R), inducing vasoconstriction and raising blood pressure, whereas Ang 1-7 binds to AT2R, leading to vasodilatation and lowering blood pressure\textsuperscript{11,12}.

As the renin-angiotensin system orchestrates blood pressure and kidney function, ACE inhibitors and AT1R blockers are widely used in hypertensive and cardiac patients\textsuperscript{13}. In this context, the chronic use of ACE inhibitors or AT1R antagonists may be of particular relevance for SARS-CoV-2-infected patients, as they may alter the dynamics of ACE-2 expression and thus increase susceptibility.
It has already been demonstrated by using hypertensive rat models that AT1R blockade elevates ACE-2 expression. Treatment with losartan and lisinopril, either alone or in association, significantly increases ACE-2 mRNA in cardiomyocytes of rats. This is associated with higher Ang 1-7 plasma concentrations\textsuperscript{14}. This was corroborated by analyzing heart tissue from rats with myocardial infarction followed by losartan treatment\textsuperscript{15}. Consistently, olmesartan, a more effective AT1R antagonist, significantly increased both cardiac and renal expression of ACE-2 in Wistar-Kyoto rats\textsuperscript{16}. This is in agreement with the use of perindopril, an ACE inhibitor, in rats\textsuperscript{17}.

Another interesting feature of the dynamics of ACE-2 expression in tissues is its capacity to be cleaved from the cell surface by a metalloproteinase called ADAM17 (TACE)\textsuperscript{18}. The ACE-2 ectodomain is cleaved, releasing soluble ACE-2 (sACE-2), whose role has not been fully elucidated. However, it has been shown that a higher concentration of sACE-2 in the plasma correlates with a poorer prognosis after heart failure\textsuperscript{19}. Notably, sACE-2 may also be detected in the cerebrospinal fluid at higher concentrations in hypertensive patients. This has been corroborated in Nefh\textsuperscript{cre} AT1aR\textsuperscript{flox/flox} mice, implicating ACE-2 and sACE-2 in the brain to the etiology of neurogenic hypertension\textsuperscript{20}.

Further, it was demonstrated that ACE-2 may be cleaved by other mechanisms. For instance, the SARS spike protein may modulate ADAM17 expression, which in turn cleaves ACE-2 to its soluble form. This was dependent both on the cytoplasmic domain of ACE-2, as siRNA for ADAM17 or ACE-2 lacking intracellular domains abrogated the phenomenon\textsuperscript{21}. Interestingly, increased shedding of ACE-2 correlates with worsening of the disease, probably due to an increase in Ang II instead of Ang 1-7. This leads to increased vascular permeability and local inflammation. Interestingly, ACE-2 cleavage
from the cell surface may also occur on lung epithelial cells\textsuperscript{22}. Thus, cleavage of ACE-2 to sACE-2 would compromise the function of Ang 1-7 on AT2R and thus reduce pulmonary hypertension and inflammation.

Altogether, as depicted in Figure 1, these data show that modulating the renin-angiotensin axis changes ACE-2 expression levels in several tissues, especially in lung and heart tissues. Whereas the use of ACE inhibitors and AT1R antagonists seem to upregulate ACE-2 expression, facilitating viral attachment and entry, and the further presence of the virus itself or some cytokines, including TNF-\(\alpha\), leads to ACE-2 release from the cell membrane, abrogating its function to counteract Ang II.

Altogether, ACE-2 overexpression may lead to facilitating viral replication in lung tissue as well as promoting lung vascular permeability, a common feature of severe SARS-CoV-2 patients. In summary, this may greatly impact the outcome of coronavirus infection in elderly and hypertensive patients, as ACE-2 is the putative attachment and invasion receptor for coronaviruses\textsuperscript{8,23}. In fact, the use of TACE inhibitors as SARS antiviral agents has already been proposed in experimental models\textsuperscript{2,3}. 
**Figure 1**: Illustrative scheme of ACE-2 expression in the lungs. Left panel: Normal expression of ACE-2 in lung tissue interacts with SARS-CoV-2. Middle panel: Increased expression of ACE-2 in lung tissues of hypertensive patients under chronic treatment with AT1R blockers. Right panel: ADAM17 cleaves ACE-2, releasing its soluble form, sACE-2, which is increased in the presence of TNF-α. Illustration elaborated by the authors using www.biorender.com.
ANTIBODY-DEPENDENT ENHANCEMENT

Antibody-dependent enhancement (ADE) is a phenomenon by which viruses use preexisting non-neutralizing antibodies from previous exposure to invade host cells through Fc receptor-mediated internalization. This is most commonly observed during secondary dengue virus (DENV) infection, causing severe hemorrhagic disease\textsuperscript{25}. Notably, the possibility of ADE between Zika virus (ZIKV), the causative agent of ZIKV congenital syndrome\textsuperscript{26}, and DENV has been intensively debated recently \textsuperscript{27,28,29}. Moreover, ADE has been the focus of debate for Ebola\textsuperscript{30} and HIV\textsuperscript{31} infections.

During ADE, preexisting antibodies elicited by previous viral exposure are not able to neutralize viral particles during a secondary infection with any antigenic-related virus. Instead, IgG opsonized viral particles then target Fc\textsubscript{γ}R expressed on endothelial and immune cells, facilitating viral attachment and invasion into host cells. Further, after intense viral replication, endothelial cells may respond by increasing vascular permeability and allowing exudate extravasation and bleeding. On the other hand, monocytes become highly activated and may undergo a cytokine storm\textsuperscript{32}.

In the context of SARS-CoV-2, it is plausible to think that ADE occurs. As mentioned in the introduction, elderly (>60 yo) patients are more susceptible to infection for still unknown reasons. As coronaviruses in general are very prevalent in the population worldwide, causing mild infection and flu-like symptoms, seroconversion to previous circulating coronaviruses is probably widespread. Thus, it is reasonable to infer that elderly patients, for obvious reasons, have been exposed to previous infections more times than younger subjects. This would imply a vaster repertoire of antibodies
against coronavirus epitopes produced by long-living plasma cells, which in fact has recently been shown to expand during SARS-CoV-2.

However, whether these antibodies are either neutralizers or enhancers must be further addressed. It is worth mentioning that, concerning the newly SARS-CoV-2, sequencing analysis of the viral genome isolated in Wuhan, China, indicated that mutations mainly occurred within the spike protein coding sequence, which has less than 40% identity to previously circulated coronaviruses\(^1\). These mutations may be responsible not only for the spill over from bats to humans but also for inducing ADE, as changes in spike epitopes may result in the interaction with non-neutralizing antibodies. Corroborating this, a novel epitope was mapped, which is lacking in previous isolates\(^3^3\).

As COVID-19 is not a hemorrhagic disease, it is probable that ADE, if present, is not mediated by endothelial cells. However, it has already been shown that lung epithelial cells highly express FcγRIIa\(^3^4\). Moreover, immune cells, including monocytes and dendritic cells, greatly express this receptor. These populations, especially monocytes, greatly account for the inflammatory infiltrate in the lungs during pneumonia, which is consistent with the transient lymphopenia observed in patients, as circulating cells may migrate to the lungs. Conversely, lung imaging of severe COVID-19 patients greatly shows lung opacity, which is consistent with edema and cellular infiltrate\(^5\). Thus, it is feasible that lung-infiltrating monocytes expressing FcγR greatly favor SARS-CoV-2 replication in lung tissue, accounting for the greater susceptibility of elderly patients.

Corroborating this hypothesis, young individuals and children do not account for the risk group for severe disease, which greatly differs from influenza virus\(^7\), for instance.
In the context of ADE, it is plausible to think that children, as they had less or no exposure to previous circulating coronaviruses, bear a very restricted repertoire of IgG or only low-affinity IgM, which is not capable of inducing ADE. In this context, mapping IgGs complementary determining regions (CDRs) from young and elderly individuals would be of great importance not only to find neutralizing antibodies but also to address this hypothesis.

Additionally, previous studies of MERS and SARS have already brought attention to the possibility of ADE. A recent work published by Wan Y et al 2020 elucidated the mechanism by which monoclonal antibodies (mAbs) induce ADE in human cells. Interestingly, mAbs that target the receptor binding domain (RBD) of SARS and MERS spike proteins induced conformational changes in the protein that favor an interaction with cell receptor dipeptidyl peptidase 4 (DPP4), the described receptor for MERS. Moreover, immunocomplexes also promoted viral entry. However, increasing concentrations of antibodies block viral invasion, as RBDs became inaccessible.

This is consistent with previous findings by Wang F et al 2014. The promonocytic cell line HL-CZ that expresses both ACE-2 and FcγR was infected with SARS-CoV in the presence of increasing concentrations of anti-sera. The data demonstrated that whereas higher concentrations neutralized the virus, low concentrations induced ADE. More interestingly, anti-sera also recognized spike protein-related antigens.

Thus, as indicated in Figure 2, circulating antibodies, instead of neutralizing the current circulating SARS-CoV-2, may bind to viral particles and thus promote Fc-mediated internalization by lung epithelial cells and infiltrating monocytes, contributing to the worsening of COVID-19. These are the most recent and mechanistic studies on
ADE of coronaviruses thus far. Although the data are consistent, whether the phenomenon of ADE is observed in severe COVID-19 patients still has to be determined.

**Figure 2:** Illustrative scheme of ADE during SARS-CoV-2 infection. Left panel: First infection and lack of preexisting antibodies allow viral particles to interact with ACE-2. Middle panel: Preexisting low-affinity antibodies or antibodies at sub-optimal concentrations bind to viral particles and facilitate the internalization mediated by FcRs either on epithelial or immune cells. Right panel: Neutralizing IgG antibodies elicited after vaccination or neutralizing IgM that do not mediate enhancement bind to viral particles. Illustration elaborated by the authors using www.biorender.com.

**CONCLUSIONS**

SARS-CoV-2 is the newest threat to human health and needs emergency actions from governments and public health agencies worldwide, especially due to its pandemic potential, as recently declared by the World Health Organization (WHO). In this context,
it is essential to rapidly and deeply address all the possibilities concerning the severity of infection, especially in the elderly population, which accounts for approximately 10-12% of the mortality rate. Here, we present several relevant aspects that may contribute to the increased susceptibility of COVID-19 in the aforementioned population. We believe that i) increased expression of ACE-2 in hypertensive patients under treatment with ACE inhibitors and AT1R blockers and ii) previous exposure to circulating coronaviruses with low neutralizing capacity to SARS-CoV-2 may greatly contribute to the increased susceptibility of elderly patients. To determine whether these hypotheses are correct, further investigations are needed, not only to better understand the etiology of the current SARS-CoV-2 infection but also to be better prepared in the possibility of future epidemics.

REFERENCES


4. WHO. World Health Organization - Situation Reports. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-


34. The Protein Cell Atlas Webpage Available from:
https://www.proteinatlas.org/ENSG00000143226-FCGR2A/tissue.