REVIEW

The renin–angiotensin–aldosterone system: Role in pathogenesis and potential therapeutic target in COVID-19

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Abstract

Coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 novel coronavirus, has spread worldwide causing high fatality rates. Neither a vaccine nor specific therapeutic approaches are available, hindering the fight against this disease and making better understanding of its pathogenesis essential. Despite similarities between SARS-CoV-2 and SARS-CoV, the former has unique characteristics which represent a great challenge to physicians. The mechanism of COVID-19 infection and pathogenesis is still poorly understood. In the present review, we highlight possible pathways involved in the pathogenesis of COVID-19 and potential therapeutic targets, focusing on the role of the renin–angiotensin–aldosterone system.

KEYWORDS
angiotensin-converting enzyme, COVID-19, inflammation, SARS-CoV-2, therapy

1 | INTRODUCTION

In December 2019, an atypical viral pneumonia caused by a novel coronavirus was identified in Wuhan, China (https://www.who.int/). Within months, the disease, later named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO), had spread worldwide and become a global health emergency.[1] According to WHO, as of June 09, 2020, the number of confirmed cases was over 7,039,918 and the number of deaths more than 404 396. (https://www.who.int/). United Kingdom, Spain, and Italy followed by France, have the highest number of cases in Europe, while the United States represents the epicenter of the disease in the American continent. Some countries, such as China, Germany, and Denmark, are exhibiting a progressive decline in cases, with hopes that the pandemic has not only peaked but also been controlled in these territories (https://covid19.who.int).

The median age of infected individuals who need hospitalization ranges from 49 to 56; however, patients who need intensive care unit (ICU) care have been significantly older, with a median age of approximately 66 years.[2-4] Moreover, individuals with chronic co-morbidities such as hypertension and diabetes are at the highest risk of poor outcomes when infected.[5,6] The clinical picture of COVID-19 ranges from asymptomatic to severe respiratory failure. The main symptoms are fever, fatigue, and cough; patients can be classified as mild, severe, and critical according to clinical presentation.[7] Different pathophysiological pathways have been identified and explored, but there is no clear evidence of protective or risk factors for SARS-CoV-2 infection. In the present review, we highlight possible pathways involved in the pathogenesis of COVID-19, with focus on the role of the renin–angiotensin–aldosterone system (RAAS).

2 | ACE2 IN SARS-COV-2 INFECTION

A key structural component of all coronaviruses is the envelope-anchored spike (S) protein, which enables the virus to bind to receptors on the host cell (Figure 1).[8,9] According to Zhou et al, SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptor to invade and infect cells.[10] Hoffmann et al further suggested that a host cell protease is necessary to allow virus fusion.[11]

ACE2 is a type I integral monocarboxypeptidase with 46% homology to ACE protein sequence.[12,13] Structurally, ACE2 has a catalytic metalloprotease domain, a signal peptide, and a trans-membrane domain.[12] Its extracellular catalytic domain contains a substrate binding region and zinc-binding site critical for its activity. Usually, its cleavage site is preceded by a X-Pro or Pro-X-Pro motif.[14] ACE2 cleavages Ang II at C-terminal domain removing phenylalanine (^{7}Pro-^{8}Phe) forming angiotensin (1-7) (Ang(1-7)).

ACE2 is highly expressed in lung epithelial and endothelial cells, which explains the primary occurrence of respiratory system dysfunction during COVID-19 infection.[15] Among lung cells, it has been observed that virus-related genes were more likely expressed in type 2 lung epithelial cells, which may explain the severe alveolar
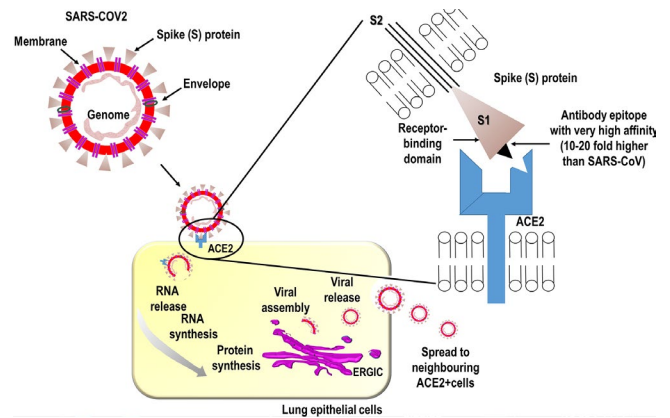

FIGURE 1 Structural proteins of SARS-COV-2: spike (S), envelope (E), and matrix (M), as well as nucleocapsid (N) proteins 3-5. The S protein is divided into two subunits, S1 and S2. The S1 domain attaches to cells through angiotensin-converting enzyme 2 (ACE2). The resulting virus-ACE2 complex is translocated into the cell and a host protease cleaves the S2 domain, which releases the viral genome into the cytoplasm. In the cytoplasm, the viral genome is translated into replicase polyproteins that drive RNA synthesis and replication. Virus structural and nonstructural proteins are then synthesized using intracellular machinery. These proteins bud into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC); new viral particles are then assembled and released to infect new target cells

damage seen after infection. Nevertheless, ACE2 is also expressed in the kidney, heart, tongue, ileum, and esophagus, thus explaining the presence of nonrespiratory symptoms.[16,17]

As noted above, binding of SARS-CoV-2 to ACE2 is mediated by the S protein.[11,18] The SARS-CoV-2 S protein has approximately 76% homology with that of SARS-CoV, the virus which caused the 2002-2004 severe acute respiratory syndrome outbreak.[19] He et al observed that a mutation leading to substitution of arginine residue 44 of the SARS-CoV S protein by alanine (R44A) abolished its binding to ACE2.[20] Structural data have demonstrated that the SARS-CoV-2 receptor binding domain (RBD), located in the S1 domain of the spike protein, interacts with the catalytic domain of ACE2.[21-24]

Importantly, SARS-CoV-2 carries mutation in the spike protein RBD that could confer higher affinity for ACE2 when compared to the SARS-CoV spike protein, including Val404 to Lys307 and Arg426 to Asn439, due to salt bridge and van der Waals contact, respectively, as shown by cryo-EM interface comparison.[22,24] This could explain, at least in part, the higher transmissibility of SARS-CoV-2.[21-23]

Upon engagement of ACE2, SARS-CoV-2 S protein is primed by the transmembrane serine protease 2 (TMPRSS2) in two distinct subunits, S1 and S2, a step essential for efficient virus replication.[11] Importantly, despite the suggestion that SARS-CoV could fuse with the host plasma memrane after ACE2 binding,[25] it has been demonstrated that viral entry is also mediated by clathrin-independent and caveolin-independent endocytosis,[26] involved in lipid raft formation crucial for SARS-CoV internalization.[26] However, the molecular mechanism involved in SARS-CoV-2 infection has not been completely determined. An important clue

comes from the observation that endosomal alkalization induced by ammonium chloride inhibited SARS-CoV-2 replication.[11] Ou et al,[19] using in vitro infection of HEK293/hACE2 cells (expressing ACE2) with SARS-CoV-2 S pseudovirions, observed that virus infection required the cell surface endocytosis. In agreement with this finding, it has been observed that drugs such as chloroquine, which halt the progression from early endosome to lysosome, decrease the viral replication rate.[27] These observations have sparked great interest in the potential utility of these drugs for treatment of COVID-19, although no scientific evidence to support this has been obtained to date.

3 | RAAS AND ITS RELATIONSHIP WITH COVID-19: FRIEND OR FOE?

The identification of ACE2 as the receptor of SARS-CoV-2 places the RAAS at the centre of COVID-19 pathogenesis. Furthermore, since this protease is involved in angiotensin peptide metabolism, which regulates the immune system, it is plausible to suggest that other components of the RAAS might play roles in COVID-19 pathogenesis.

The RAAS is a regulatory proteolytic cascade involved in a variety of physiological functions in different organs, including the heart, kidneys, and lungs.[28] The central axis involves the production of angiotensin II (Ang II) by proteolytic cleavage of angiotensin I (Ang I), promoted by angiotensin-converting enzyme (ACE). Ang II elicits responses through two G protein-coupled receptors (GPCR)—AT1 (AT1R) and AT2 (AT2R).[29,30] AT1R signaling is associated with effects that include vasoconstriction, a pro-inflammatory response, and anti-natriuresis.[29-31] In contrast, AT2R signaling is reported to oppose AT1R effects, promoting vasodilation, an anti-inflammatory response, and natriuresis.[29,30,32,33]

ACE2 preferentially cleaves Ang II, producing Ang (1-7)[34,35] and, to a lesser extent, cleaves Ang I to form Ang (1-9), a peptide whose role remains unknown.[12] The mechanisms mediating Ang (1-7) effects involve a complex network mediated by another GPCR—the MAS receptor (MASR)—and AT2R.[13,36-38] Other peptides whose functions are less known, such as alamandine, might play a significant role in the RAAS, but their possible role in COVID-19 will not be discussed in the present review.[39-41]

There is now a consensus that the final effect of the RAAS cascade depends on a fine equilibrium between the ACE/Ang II/AT1R and ACE2/Ang (1-7)/MASR/AT2R axes (Figure 2); their uncoupling is implicated in the pathogenesis of different lung diseases, including viral infection by respiratory syncytial virus and SARS-CoV,[18,42] pulmonary hypertension,[43,44] and acute lung injury.[45,46] Accordingly, it has been previously shown that the SARS-CoV S protein decreases ACE2 protein expression in the lung, increases Ang II levels, and enhances lung injury in an Ang II/AT1R-dependent manner.[18] These observations raise extremely relevant questions—is RAAS involved in the pathogenesis of COVID-19? How? Is this a targetable therapeutic opportunity?
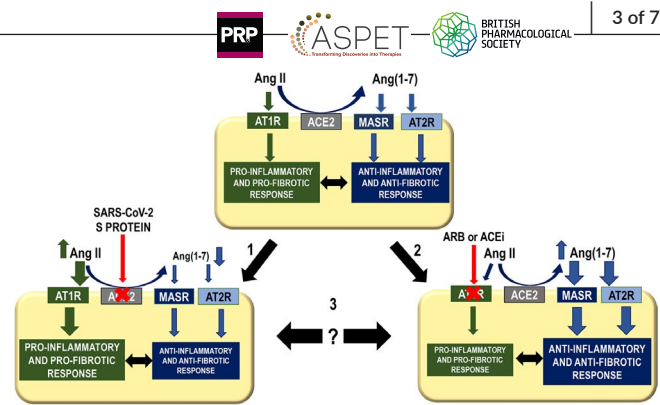

FIGURE 2 A fine balance between the ACE/Ang II/AT1R and ACE2/Ang (1-7)/MASR axes of the renin–angiotensin–aldosterone system is observed in physiological conditions. (1) During SARS-CoV-2 infection, ACE2 is downregulated, promoting the ACE/Ang II/AT1R axis and, consequently, a proinflammatory and profibrotic response. (2) Patients with chronic diseases such as hypertension and diabetes are often treated with ARB/ACEi. These medications upregulate the ACE2/Ang (1-7)/MASR axis, which in turn reduces inflammation and fibrosis signals. (3) How these different conditions are correlated, however, remains to be explored

RAAS dysregulation is a well-known mechanism involved in the genesis and progression of several comorbidities known to enhance COVID-19 susceptibility, including hypertension and diabetes.[47-49] Indeed, angiotensin AT1 receptor blockers (ARBs) and ACE inhibitors (ACEi) are the first-line therapeutic strategies used to decrease cytokine production and halt end-organ damage in both conditions.[50-53] These positive effects have been also observed after recombinant ACE2 treatment and Ang (1-7) administration.[54] These observations suggest the possibility of a therapeutic opportunity for the use of ARB and ACEi to attenuate patient responses to SARS-CoV-2 infection, in combination with viral-targeted therapy.

On the other hand, some authors have observed that severe COVID-19 cases were associated with chronic use of ARB and ACEi. One possible mechanism might be the upregulation of ACE2 expression induced by these treatments, as previously described,[55,56] although lung ACE2 expression in this setting has yet to be examined. This preliminary hypothesis led to a debate on whether ARB and ACEi should be discontinued in order to decrease COVID-19 susceptibility.[57] However, a consensus quickly emerged that these therapies should be maintained, since there is not enough evidence to support withdrawal of established therapeutic strategies against hypertension and diabetes (https://professional.heart.org/professional/ScienceNews/UCM_505836_HFSAACCAHA-statement-addresses-concerns-re-using-RAAS-antagonists-in-COVID-19.jsp,https://www.escardio.org/Councils/Council-on-Hypertension-(CHT)/News/position-statement-of-the-esc-council-on-hypertension-on-ace-inhibitors-and-ang). This position seeks both to maintain comorbidities under control and to avoid secondary events during COVID-19 infection. Corroborating this recommendation, Zhang et al found in a retrospective study that COVID-19-related mortality was significantly lower in patients treated with ARB/ACEi than in those not treated with these drugs.[58]

Taken together, the evidence suggests a time-dependent correlation between the RAAS and COVID-19 pathogenesis (Figure 3). Initially, treatment with ARB and/or ACEi promotes an increase in ACE2/Ang (1-7)/MASR axis activity, associated with an anti-inflammatory profile. At this stage, SARS-CoV-2 binding and infection is promoted. As the course of infection progresses, the ACE2/Ang (1-7)/MASR axis is downregulated, leading to internalization of ACE2 and a consequent reduction in its expression. This ACE2 downregulation enhances the ACE/Ang II/AT1R axis, which may explain several pathological events observed during COVID-19 progression, such as lung edema, immune cell infiltration within the lung, and an intense proinflammatory response.

Importantly, vascular damage has been highlighted as a hallmark of COVID-19 severity. Ackermann et al[59] observed that marked endothelial injury, with signs of viral replication, accompanied by microangiopathy and thrombosis that are associated to severe COVID-19. Indeed, it has been described that Ang II promotes vascular leakage, endothelial release of reactive oxygen species (ROS), and endothelial cell proliferation that is correlated with endothelial dysfunction.[60-63] Furthermore, ACE2 and TRMPS2 are expressed in endothelial cells, indicating possible productive replication of SARS-CoV-2 in this compartment.[64] Based on these observations, it is plausible to postulate that SARS-CoV-2 infection and replication within lung microvasculature might contribute to initial increase in viral load. Therefore, increased Ang II/AT1R axis further promotes COVID-19 progression to severe and fatal cases through, at least in part, induction of endothelium dysfunction and vascular permeability and, consequently, edema. The actual contribution of SARS-CoV-2 replication within the endothelial cells for COVID-19 outcome should be further investigated. Other possible link between RAS and vascular damage could be the Kallikrein Kinin System. It is well
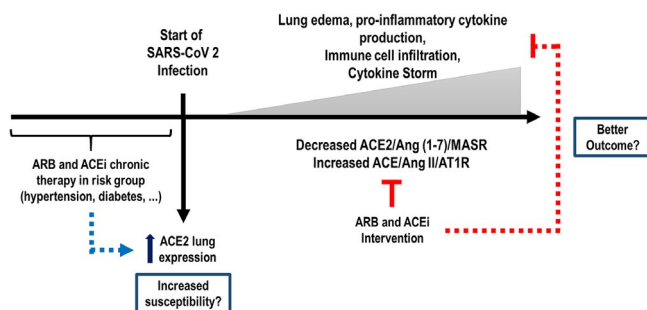
known that ACE2 breaks down bradykinin into desArg9-bradykinin, which, through B1 receptor activation, is involved in endothelial dysfunction.[65]

However, one fundamental question remains—why are hypertensive patients more susceptible to severe COVID-19? One possibility comes from the observation that an overactivation of proinflammatory response triggered by SARS-CoV-2, the so-called cytokine storm, is associated with worse outcomes.[2,4,66] Huang et al showed that critically ill COVID-19 patients have high levels of serum proinflammatory cytokines such as IL-1β, IFN-γ, IP-10, and MCP-1, which are associated with disease severity.[7] Additionally, Qin et al showed that the number of helper, suppressor, and regulatory T cells is decreased.[67] The cytokine storm leads to host cell damage, resulting in alveolar edema and pulmonary fibrosis as well as less-known heart and kidney injuries.[28]

The evidence suggests that a low-grade proinflammatory response could be implicated in hypertension having a causative relationship with changes observed in peripheral vascular resistance and the neural, cardiac, and renal systems.[68] Ang II-induced hypertension is known to involve polarization of CD4$^+$ T cells toward the Th1 and Th17 phenotypes to the detriment of a Th2 phenotype, and involves an increase in IFN-γ, IL-6, and IL-17.[69-71] Furthermore, cytotoxic CD8$^+$ T cells play an important role in this process, as their levels are increased in the kidney,[72] a possible target organ of SARS-CoV-2. Interestingly, the innate immune response, which involves monocytes, macrophages, granulocytes, and dendritic cells, seems to be involved in endothelial dysfunction observed in Ang II-induced hypertension.[68]

Based on these observations, we may infer that the association between hypertension and worse prognosis in COVID-19 could be due to a preexisting proinflammatory state observed in hypertensive patients. Once a hypertensive patient is infected with SARS-CoV-2, sensitization of the immune system toward an overactivation of the inflammatory response could be associated with subsequent development of cytokine storm.

4 | THERAPEUTIC OPTIONS FOR SARS-COV-2 TARGETING THE RAAS

The pathophysiological insights described above open up new possibilities for therapeutic proposals as follows: (a) use of protease inhibitors to block S protein priming. In this context, it has been proposed that the clinically approved serine protease inhibitor camostat mesylate might decrease SARS-CoV-2 entry in vitro.[11] Clinical trials testing of the effect of camostat on COVID-19 are now ongoing (NCT04321096); (b) use of chloroquine/hydroxychloroquine do inhibit viral replication, as demonstrated in different preclinical models.[73-75] In the last months, the use of chloroquine/hydroxychloroquine has been extensively debated and further randomized, placebo-controlled, clinical studies are required to clarify the benefits of chloroquine/hydroxychloroquine therapy for COVID-19 patients. The purported molecular

FIGURE 3 COVID-19 disease pathogenesis and a proposal for ARB/ACEi-based intervention. Schematic diagram of the time-dependent correlation between RAAS activity and COVID-19 pathogenesis. Risk-group patients receiving ARB/ACEi likely overexpress ACE2, which could at least partly explain their higher susceptibility to severe illness. However, experimental evidence suggests that, during the course of SARS-CoV-2 infection, there is upregulation of the ACE/Ang II/AT1R axis to the detriment of the ACE2/Ang (1-7)/MASR axis. This could account for the lung edema, immune cell infiltration within the lung, and cytokine storm observed in infected patients. In this stage of the disease, ARB/ACEi could represent a therapeutic opportunity to halt the host response to SARS-CoV-2 infection

mechanism involves alkalization of endosomal pH and subsequent inhibition of membrane fusion[76]; (c) based on the homology between SARS-CoV and SARS-CoV-2 S protein, antisera from immunized patients, ACE2 neutralizing antibody, or recombinant ACE2 have been proposed as possible inhibitors of viral engagement on epithelial cells.[11,77] Indeed, a monoclonal antibody against the receptor binding domain (RBD) of SARS-CoV-2, obtained from B cells of immunized COVID-19 patients, has been shown to block the SARS-CoV-2 S protein/ACE2 interaction.[15] Moreover, structural data on this interaction have highlighted design strategies for the development of molecular inhibitors [21-24]; d) based on the observation that ARBs and/or ACEi could blunt the proinflammatory response in different chronic degenerative diseases, it is plausible to suggest their use in severe cases of COVID-19, where cytokine storm is one of the main concerns. Accordingly, Meng et al[78] observed that Chinese hypertensive patients treated with ACEi or ARB who were stricken with COVID-19 had a lower rate of progression to severe illness, with a trend toward lower IL-6 plasma levels. A clinical trial of treatment with losartan is underway (NCT04328012).

5 | CONCLUSION

COVID-19 is spreading at an alarming rate worldwide. Although SARS-CoV-2 has structural and functional similarities with SARS-CoV, new attributed capabilities of SARS-CoV-2 have been discovered. The absence of a vaccine or specific treatment and the intense immune response triggered by SARS-CoV-2 have been associated with the large number of infections and deaths. Here we highlighted a particularly important pathway—the renin–angiotensin–aldosterone system—whose role in COVID-19 pathogenesis must be further understood in order to develop effective therapeutic strategies for COVID-19.

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REFERENCES

1. The Lancet Infectious Diseases. COVID-19, a pandemic or not? *Lancet Infect Dis.* 2020;20:383-https://doi.org/10.1016/S1473-3099(20)30180-8
2. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2). *Science.* 2020;368:489-493. https://doi.org/10.1126/science.abb3221
3. Meo SA, Alhowikan AM, Al-Khlaiwi T, et al. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. *Eur Review Med Pharmacol Sci.* 2020;24:2012-2019. https://doi.org/10.26355/eurrev_202002_20379
4. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* 2020;323:1061-https://doi.org/10.1001/jama.2020.1585
5. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med.* 2020;8:e21. https://doi.org/10.1016/S2213-2600(20)30116-8
6. Lian J, Jin X, Hao S, et al. Analysis of epidemiological and clinical features in older patients with Coronavirus Disease 2019 (COVID-19) outside Wuhan. *Clin Infect.* https://doi.org/10.1093/cid/ciaa242
7. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395:497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
8. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV — a target for vaccine and therapeutic development. *Nat Rev Microbiol.* 2009;7:226-236. https://doi.org/10.1038/nrmicro2090
9. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* 2020;367:1260-1263. https://doi.org/10.1126/science.abb2507
10. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* 2020;579:270-273. https://doi.org/10.1038/s41586-020-2012-7
11. Hoffmann M, Kleine-Weber H, Schroeder S, CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* 2020;181:271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052
12. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res.* 2000;87:1-9. https://doi.org/10.1161/01.RES.87.5.e1
13. Jiang F, Yang J, Zhang Y, et al. Angiotensin-converting enzyme 2 and angiotensin 1–7: novel therapeutic targets. *Nat Rev Cardiol.* 2014;11:413-426. https://doi.org/10.1038/nrcardio.2014.59
14. Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ. Angiotensin-converting enzyme-2 (ACE2): comparative modeling of the active site, specificity requirements, and chloride dependence. *Biochemistry.* 2003;42:13185-13192. https://doi.org/10.1021/bi035268s
15. Chen X, Li R, Pan Z, et al. Human monoclonal antibodies block the binding of SARS-CoV-2 spike protein to angiotensin converting enzyme 2 receptor. *Cell Mol Immunol.* 2020;17:647-649. https://doi.org/10.1038/s41423-020-0426-7
16. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci.* 2020;12:8. https://doi.org/10.1038/s41368-020-0074-x
17. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med.* 2020;14:185-192. https://doi.org/10.1007/s11684-020-0754-0
18. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. *Nat Med.* 2005;11:875-879. https://doi.org/10.1038/nm1267
19. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun.* 2020;11:1620. https://doi.org/10.1038/s41467-020-15562-9
20. He L, Ding Y, Zhang Q, et al. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J Pathol* 2006;210:288-297. https://doi.org/10.1002/path.2067

21. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*. 2020;581:215-220. https://doi.org/10.1038/s41586-020-2180-5

22. Shang J, Ye G, Shi K, et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature*. 2020;581:221-224. https://doi.org/10.1038/s41586-020-2179-y

23. Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell Mol Immunol*. 2020;17:613-620. https://doi.org/10.1038/s41423-020-0400-4

24. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*. 2020;367:1444-1448. https://doi.org/10.1126/science.abb2762

25. Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P. Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. *Proc Natl Acad Sci*. 2004;101:4240-4245. https://doi.org/10.1073/pnas.0306446101

26. Wang H, Yang P, Liu K, et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res*. 2008;18:290-301. https://doi.org/10.1038/cr.2008.15

27. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov*. 2020;6:16. https://doi.org/10.1038/s41421-020-0156-0

28. Seva Pessoa B, van der Lubbe N, Verdonk K, Roks AJ, Hoorn EJ., Danser AH. Key developments in renin–angiotensin–aldosterone system inhibition. *Nat Rev Nephrol*. 2013;9:26-36. https://doi.org/10.1038/nrneph.2012.249

29. Forrester SJ, Booz GW, Sigmund CD, et al. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. *Physiol Rev*. 2018;98:1627-1738. https://doi.org/10.1152/physrev.00038.2017

30. Singh KD, Karnik SS. Angiotensin receptors: structure, function, signaling and clinical applications. *J Cell Signal*. 1. https://doi.org/10.4172/jcs.1000111

31. Landgraf SS, Wengert M, Silva JS, et al. Changes in angiotensin receptors expression play a pivotal role in the renal damage observed in spontaneously hypertensive rats. *Am J Physiol-Renal Physiol*. 2011;300:F499-F510. https://doi.org/10.1152/ajprenal.00384.2010

32. Inuzuka T, Fujioka Y, Tsuda M, et al. Attenuation of ligand-induced activation of angiotensin II type 1 receptor signaling by the type 2 receptor via protein kinase C. *Sci Rep*. 2016;6:21613. https://doi.org/10.1038/srep21613

33. Matavelli LC, Huang J, Siragy HM. Angiotensin AT(2) receptor stimulation inhibits early renal inflammation in renovascular hypertension. *Hypertension* 2011;57:308-313. https://doi.org/10.1161/HYPERTENSIONAHA.110.164202

34. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. *J Biol Chem*.2000;275:33238-33243.https://doi.org/10.1074/jbc.M002615200

35. Vickers C, Hales P, Kaushik V, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem*. 2002;277:14838-14843. https://doi.org/10.1074/jbc.M200581200

36. Bosnyak S, Jones ES, Christopoulos A, Aguilar MI, Thomas WG, Widdop RE. Relative affinity of angiotensin peptides and novel ligands at AT1 and AT2 receptors. *Clini Sci*. 2011;121:297-303. https://doi.org/10.1042/CS20110036

37. Lara LS, Bica RB, Sena SL, et al. Angiotensin-(1–7) reverts the stimulatory effect of angiotensin II on the proximal tubule Na(+)-ATPase activity via a A779-sensitive receptor. *Regul Pept*. 2002;103:17-22. https://doi.org/10.1016/s0167-0115(01)00322-6

38. Santos RA, Simoes e Silva AC, Maric C, et al. Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci*. 2003;100:8258-8263. https://doi.org/10.1073/pnas.1432869100

39. McKinney CA, Fattah C, Loughrey CM, Milligan G, Nicklin SA. Angiotensin-(1–7) and angiotensin-(1–9): function in cardiac and vascular remodelling. *Clin Sci*. 2014;126:815-827. https://doi.org/10.1042/CS20130436

40. Villela DC, Passos-Silva DG, Santos RA. Alamandine. *Curr Opin Nephrol Hypertens*. 2014;23:130-134. https://doi.org/10.1097/01.mnh.0000441052.44406.92

41. Yugandhar VG, Clark MA. Angiotensin III: a physiological relevant peptide of the renin angiotensin system. *Peptides*. 2013;46:26-32. https://doi.org/10.1016/j.peptides.2013.04.014

42. Gu H, Xie Z, Li T, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. *Sci Rep*. 2016;6:19840. https://doi.org/10.1038/srep19840

43. Li G, Liu Y, Zhu Y, et al. ACE2 activation confers endothelial protection and attenuates neointimal lesions in prevention of severe pulmonary arterial hypertension in rats. *Lung*. 2013;191:327-336. https://doi.org/10.1007/s00408-013-9470-8.

44. Rondelet B, Kerbaul F, Van Beneden R, et al. Prevention of pulmonary vascular remodeling and of decreased BMPR-2 expression by losartan therapy in shunt-induced pulmonary hypertension. *Am J Physiol-Heart Circ Physiol*. 2005;289:H2319-H2324. https://doi.org/10.1152/ajpheart.00518.2005

45. Chen LN, Yang XH, Nissen DH, et al. Dysregulated renin-angiotensin system contributes to acute lung injury caused by hind-limb ischemia-reperfusion in mice. *Shock*. 2013;40:420-429. https://doi.org/10.1097/SHK.0b013e3182a6953e

46. Li Y, Zeng Z, Cao Y, et al. Angiotensin-converting enzyme 2 prevents lipopolysaccharide-induced rat acute lung injury via suppressing the ERK1/2 and NF-kappaB signaling pathways. *Sci Rep*. 2016;6:27911. https://doi.org/10.1038/srep27911

47. Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. *BMJ*. 2020;368:m1198. https://doi.org/10.1136/bmj.m1198

48. Ribeiro-Oliveira A Jr, Nogueira AI, Pereira RM, Boas WW, Dos Santos RA, Simoes e Silva AC. The renin-angiotensin system and diabetes: an update. *Vasc Health Risk Manage*. 2008;4:787-803.

49. Santos RA. Angiotensin-(1–7). *Hypertension*. 2014;63:1138-1147. https://doi.org/10.1161/HYPERTENSIONAHA.113.01274

50. ADA. Standards of medical care in diabetes-2018 abridged for primary care providers. *Clin Diabetes*. 2018;36:14-37. https://doi.org/10.2337/cd17-0119

51. Benicky J, Sanchez-Lemus E, Honda M, et al. Angiotensin II AT1 receptor blockade ameliorates brain inflammation. *Neuropsychopharmacology*. 2011;36:857-870. https://doi.org/10.1038/npp.2010.225

52. Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J Hum Hypertens*. 2007;21:20-27. https://doi.org/10.1038/sj.jhh.1002101

53. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *J Hypertens*. 2018;36:1953-2041. https://doi.org/10.1097/HJH.0000000000001940

54. Santos RAS, Sampaio WO, Alzamora AC, et al. The ACE2/angiotensin-(1–7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1–7). *Physiol Rev*. 2018;98:505-553. https://doi.org/10.1152/physrev.00023.2016

55. Ferrario CM, Jessup J, Chappell MC, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor

blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111:2605-2610. https://doi.org/10.1161/CIRCULATIONAHA.104.510461

56. Li XC, Zhang J, Zhuo JL. The vasoprotective axes of the renin-angiotensin system: Physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol Res*. 2017;125:21-38. https://doi.org/10.1016/j.phrs.2017.06.005

57. Kuster GM, Pfister O, Burkard T, et al. SARS-CoV2: should inhibitors of the renin-angiotensin system be withdrawn in patients with COVID-19? *Eur Heart J*. 2020;41:1801-1803. https://doi.org/10.1093/eurheartj/ehaa235

58. Zhang P, Zhu L, Cai J, et al. Association of inpatient use of angiotensin converting enzyme inhibitors and angiotensin ii receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ Res*. 2020;126:1671-1681. https://doi.org/10.1161/CIRCRESAHA.120.317134

59. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med*. 2020. https://doi.org/10.1056/NEJMoa2015432

60. Bodor C, Nagy JP, Végh B, et al. Angiotensin II increases the permeability and PV-1 expression of endothelial cells. *Am J Physiol Cell Physiol*. 2012;302:C267. https://doi.org/10.1152/ajpcell.00138.2011

61. Guo S, Som AT, Arai K, Lo EH. Effects of angiotensin-II on brain endothelial cell permeability via PPARalpha regulation of para- and trans-cellular pathways. *Brain Res*. 2019;1722:146353. https://doi.org/10.1016/j.brainres.2019.146353

62. Tamarat R, Silvestre JS, Durie M, Levy BI. Angiotensin II angiogenic effect in vivo involves vascular endothelial growth factor- and inflammation-related pathways. *Lab Invest*. 2002;82:747-756. https://doi.org/10.1097/01.lab.0000017372.76297.eb

63. Xiao X, Zhang C, Ma X. Angiotensin-(1–7) counteracts angiotensin II-induced dysfunction in cerebral endothelial cells via modulating Nox2/ROS and PI3K/NO pathways. *Exp Cell Res*. 2015;336:58-65. https://doi.org/10.1016/j.yexcr.2015.06.010

64. Muss C, Luecken MD, Eraslan G, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. *bioRxiv*. 2020; https://doi.org/10.1101/2020.04.19.049254

65. van de Veerdonk FL, Netea MG, van Deuren M, et al. Kallikrein-kinin blockade in patients with COVID-19 to prevent acute respiratory distress syndrome. *Elife*. 2020;9:e57555.

66. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol*. 2017;39:529-539. https://doi.org/10.1007/s00281-017-0629-x

67. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis*. 2020;ciaa248. https://doi.org/10.1093/cid/ciaa248

68. Wenzel U, Turner JE, Krebs C, Kurts C, Harrison DG, Ehmke H. Immune mechanisms in arterial hypertension. *J Am Soc Nephrol*. 2016;27:677-686. https://doi.org/10.1681/ASN.2015050562

69. Kamat NV, Thabet SR, Xiao L, et al. Renal transporter activation during angiotensin-II hypertension is blunted in interferon-gamma-/- and interleukin-17A-/- mice. *Hypertension*. 2015;65:569-576. https://doi.org/10.1161/HYPERTENSIONAHA.114.04975

70. Schrader LI, Kinzenbaw DA, Johnson AW, Faraci FM, Didion SP. IL-6 deficiency protects against angiotensin II–induced endothelial dysfunction and hypertrophy. *Arterioscler Thromb Vasc Biol*. 2007;27:2576-2581. https://doi.org/10.1161/ATVBAHA.107.153080

71. Zhang J, Patel MB, Griffiths R, et al. Tumor necrosis factor-alpha produced in the kidney contributes to angiotensin II-dependent hypertension. *Hypertension*. 2014;64:1275-1281. https://doi.org/10.1161/HYPERTENSIONAHA.114.03863

72. Trott DW, Thabet SR, Kirabo A, et al. Oligoclonal CD8+ T cells play a critical role in the development of hypertension. *Hypertension*. 2014;64:1108-1115. https://doi.org/10.1161/HYPERTENSIONAHA.114.04147

73. Delvecchio R, Higa LM, Pezzuto P, et al. Chloroquine, an endocytosis blocking agent, inhibits Zika virus infection in different cell models. *Viruses*. 2016;8:322-https://doi.org/10.3390/v8120322

74. Keyaerts E, Li S, Vijgen L, Rysman E, Verbeeck J, Van Ranst M. Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. *Antimicrob Agents Chemother*. 2009;53:3416-3421. https://doi.org/10.1128/AAC.01509-08

75. Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun*. 2004;323:264-268. https://doi.org/10.1016/j.bbrc.2004.08.085

76. Al-Bari MAA. Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. *Pharmacol Res Perspect*. 2017;5:e00293

77. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin Sci*. 2020;134:543-545. https://doi.org/10.1042/CS20200163

78. Meng J, Xiao G, Zhang J, et al. (Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg Microbes Infec*. 2020;9:757-760.