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REVIEW

Hypertension, the renin–angiotensin system, and the risk of lower respiratory tract infections and lung injury: implications for COVID-19

European Society of Hypertension COVID-19 Task Force Review of Evidence

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Abstract

Systemic arterial hypertension (referred to as hypertension herein) is a major risk factor of mortality worldwide, and its importance is further emphasized in the context of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection referred to as COVID-19. Patients with severe COVID-19 infections commonly are older and have a history of hypertension. Almost 75% of patients who have died in the pandemic in Italy had hypertension. This raised multiple questions regarding a more severe course of COVID-19 in relation to hypertension itself as well as its treatment with renin–angiotensin system (RAS) blockers, e.g. angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). We provide a critical review on the relationship of hypertension, RAS, and risk of lung injury. We demonstrate lack of sound evidence that hypertension *per se* is an independent risk factor for COVID-19. Interestingly, ACEIs and ARBs may be associated with lower incidence and/or improved outcome in patients with lower respiratory tract infections. We also review in detail the molecular mechanisms linking the RAS to lung damage and the potential clinical impact of treatment with RAS blockers in patients with COVID-19 and a high cardiovascular and renal risk. This is related to the role of angiotensin-converting enzyme 2 (ACE2) for SARS-CoV-2 entry into cells, and expression of ACE2 in the lung, cardiovascular system, kidney, and other tissues. In summary, a critical review of available evidence does not support a deleterious effect of RAS blockers in COVID-19 infections. Therefore, there is currently no reason to discontinue RAS blockers in stable patients facing the COVID-19 pandemic.

Keywords Hypertension • Angiotensin • COVID-19 • Cardiovascular • Lung

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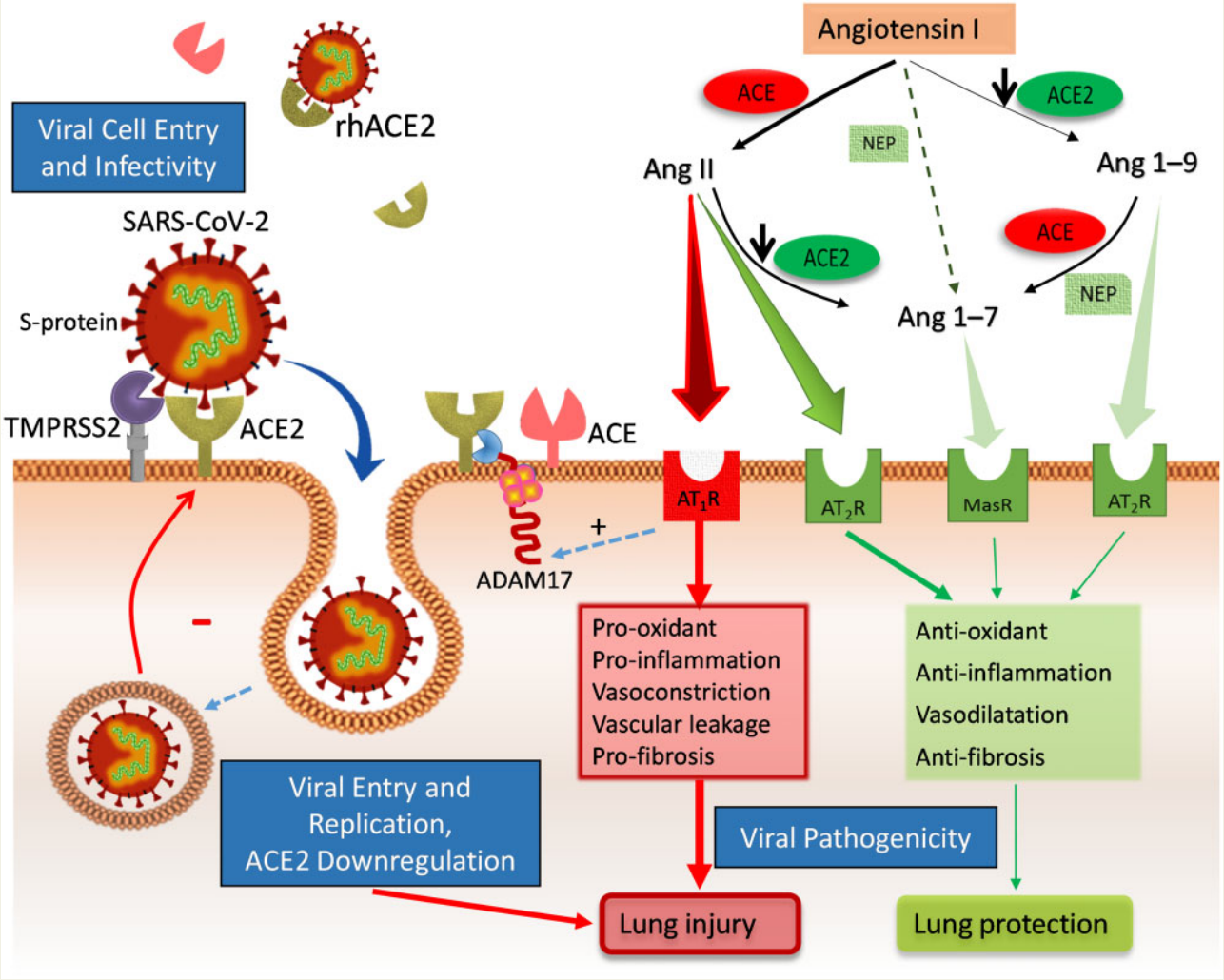
Graphical Abstract



Introduction

On 31 December 2019, a cluster of pneumonia cases of unknown origin was reported in Wuhan, Hubei Province, China. On 9 January 2020, the China Center for Disease Control and Prevention reported the causative agent as being a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease caused by SARS-CoV-2 was subsequently referred to as COVID-19.[1] On 11 March 2020, i.e. only 2 months later, the Director General of the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic.[1] At the time of writing this review, the number of confirmed COVID-19 cases is constantly increasing and it is only a matter of days until the 1 million mark is exceeded.[2] The report of an apparently high prevalence of systemic arterial hypertension (referred to as hypertension herein) in severe COVID-19 cases in an initial Chinese report on one hand,[3] and the concept that SARS-CoV-2 may use angiotensin-converting enzyme 2 (ACE2)—an enzyme potentially up-regulated by blockers of the renin–angiotensin system (RAS)[4]—as a viral entry receptor in lung cells on the other hand[5,6] has raised concerns. Indeed, given the high prevalence of hypertension in the population and the high percentage of patients receiving RAS blockers worldwide, it was speculated that these factors could contribute to the dissemination of COVID-19 and also complicate the course of the infection. Despite reassuring, consistent statements of several Hypertension Societies/Councils including the European Society of Hypertension,[7] the European Society of Cardiology Hypertension Council,[8] the International Society of Hypertension,[9] and the World Hypertension League,[10] the concern remained, as evident from subsequent reports in medical journals, social media, and the press. The scope of this article is to provide a rapid assessment and critical review of the relationship of hypertension, the RAS, and the risk of lower respiratory tract infections (LRTIs) and lung injury in the context of the COVID-19 pandemic.

Is hypertension *per se* a risk factor for lower respiratory tract infections and adverse outcome?

It has been well established that an age over 65 years is associated with an increased risk for LRTIs, including community-acquired pneumonia (CAP), and an increased rate of complications and mortality.[11,12]

Consequently, hypertension is usually observed as a frequent comorbidity in adult patients, particularly in the elderly, hospitalized for LRTI/CAP. According to a Spanish population-based study, the incidence of CAP showed a marked increase with age, but hypertension had no significant impact on the incidence of CAP.[12] In a population-based study investigating the risk of pneumonia in subjects older than 60 years in Finland, hypertension was the most common comorbidity (36.4%), followed by heart diseases and diabetes.[13] Importantly, the study indicated that age, lifestyle factors, e.g. high alcohol intake, and comorbidities including heart disease and diabetes, but not hypertension, were independently associated with increased risk of pneumonia.[13] To date, several of the available studies have shown that LRTIs are associated with an increased risk for cardiovascular events, including acute coronary syndromes and arrhythmias.[14–16] Hypertension, among other factors of higher baseline cardiovascular risk including older age and heart failure, may represent an important risk factor for cardiac complications in this setting.

Antihypertensive treatment and LRTIs

The use of individual antihypertensive drug classes has been associated with differences in the risk of LRTIs in previous studies.[17] A possible association between use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) and the risk of pneumonia has been previously analysed in a systematic review and meta-analysis.[17] Caldeira *et al.* aggregated 37 longitudinal studies of different designs including different populations in their meta-analysis and demonstrated a favourable effect of ACEIs on the risk of CAP, especially in Asian populations[17] (*Figure 1*). It is unclear whether the methodology of the studies, environmental and genetic factors, or differences in clinical management contributed to these findings.[17] Subsequently, use of ACEIs and ARBs was associated with a lower incidence of CAP compared with calcium channel blockers in a Canadian cohort of new users of antihypertensive drugs.[18] In addition, ACEIs and ARBs have been also previously linked to reduced mortality in patients with CAP[19] and a reduced risk for CAP requiring hospitalization.[20]

In summary, available studies do not provide sound evidence that hypertension is an independent risk factor for LRTI/CAP. However, they indicate that hypertension contributes to the increased occurrence of incident cardiovascular events in these patients, in agreement with the well-established risk associated with hypertension.[21,22] If anything, the use of ACEIs and ARBs may be associated with a lower incidence of pneumonia and improved outcome in patients with LRTI/CAP. This also applies to viral pneumonia, in which RAS blockers have been associated with improved pneumonia-related outcomes.[23,24]

Is hypertension associated with COVID-19 infections?

Reports from China

The prevalence of hypertension in adults is high worldwide, including China, and is particularly high in the elderly.[25,26] In China, 23.2% of the adult population aged ≥18 years are estimated to have hypertension according to the current definition and a recent nationwide survey.[26] The initially reported association between hypertension and hospitalization rates for COVID-19 in China is therefore not surprising.[3] Indeed, in a large database of 20 982 patients with diagnosed COVID-19 infections and information on underlying diseases, the proportion of self-reported hypertension was 12.6%,[27] which is very similar to the population data in China (10.9%) for self-reported hypertension.[26] In 406 deceased patients with COVID-19 infections, the overall proportion of hypertension was 39.7%,[27] and thus higher than in the overall population. However, 81% of these deceased patients were older than 60 years of age[27] and thus in agreement with the corresponding age group in the overall elderly population.[26] Hypertension may also represent a proxy for the presence of other cardiovascular risk factors such as diabetes, hypertension-mediated target organ damage, or cardiovascular complications,[28] all of which show an increasing prevalence with age. Thus, the reported association between hypertension and COVID-19 infection is likely to be confounded by age and comorbidities. Similarly, RAS blockers are frequently used to treat hypertension, and represent the backbone of therapy in hypertension and associated cardiovascular and renal comorbidities.[21] Accordingly, RAS blockers are thus expected to be frequently used in hospitalized patients as concomitant drugs. However, the use of RAS blockers in China is relatively low[26] compared with other first-line drug classes recommended in guidelines, e.g. calcium channel blockers.[28]

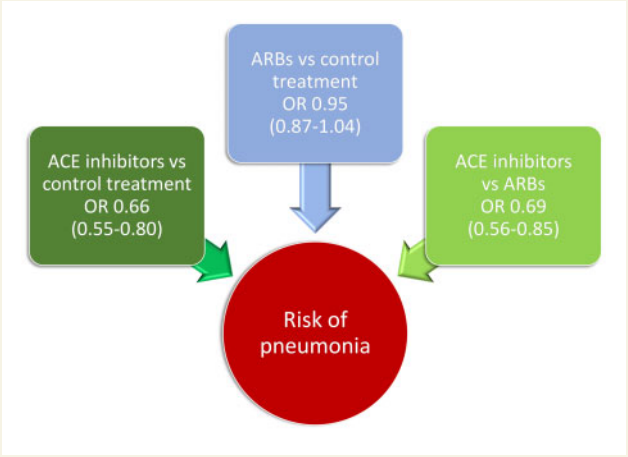


Figure 1 Risk of pneumonia with use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs): meta-analysis of 37 studies. The OR with the 95% CI in parentheses is shown.[17]

Reports from Italy

Currently, the focus of the COVID-19 epidemic has shifted from China to Europe and other regions of the world.[29] Italy was the first, and for a long time, the most severely affected European country.[30,31] Nevertheless, all other European countries appear to be in a similar situation, with just a short time lag of a couple of weeks. Italy soon had the largest number of COVID-19-related deaths after, then before, China. A high case fatality rate also characterized the COVID-19 epidemic in Italy, probably at least in part reflecting a higher proportion of old or very old patients than in other countries.[32] Along these lines, it was reported that the median age of COVID-19-positive deceased patients in Italy was 79 years and that 73% had known hypertension.[33] This proportion is even higher than in Chinese reports but still consistent with the expected high prevalence of hypertension in patients of this age range in Italy.[34] Furthermore, 30% and 17% of COVID-19-positive deceased patients were reportedly treated by ACEIs and ARBs, respectively,[33] which again does not seem excessive in this group of subjects with frequent

hypertension, and renal and cardiovascular diseases. Overall, the clinical course and outcome in patients infected with SARS-CoV-2 seems to be associated with age, with the elderly over 80 years of age being at greatest risk.[32,35,36] The latter group of patients exhibit a significant increased case fatality rate as compared with the overall infected group of patients. However, the limited data currently available do not provide firm evidence in favour of an independent association of COVID-19 infection severity with either hypertension or RAS blocker use.

Inflammatory dysregulation in hypertension as a possible pathogenetic link in COVID-19

Recent understanding of the role of immune dysregulation in hypertension[37] can provide a possible mechanistic link between immune dysregulation and a more severe course of COVID-19. Rapid deterioration in COVID-19 patients is associated with a pro-inflammatory cytokine storm. Accordingly, an increase in systemic interleukin (IL)-2, IL-6, and IL-7, granulocyte colony-stimulating factor, C-X-C motif chemokine 10 (CXCL10), chemokine (C-C motif) ligand 2 (CCL2), and tumour necrosis factor-α (TNF-α) has been observed in patients with COVID-19.[38] Interestingly, the same cytokines have been associated with the development of hypertension in experimental[39,40] and clinical observational,[37] as well as interventional, studies.[41] For example, IL-6 which appears to be strongly linked to clinical outcomes of COVID-19,[42] is one of the key cytokines regulating immune-inflammatory responses in hypertension.[43,44] Loss of lymphocytes is one of the key features of COVID-19, and a recent study in the UK Biobank population demonstrated that among white blood cells, hypertension is causally associated with lymphocytes.[45] Moreover, it was shown that CD4+, and in particular CD8+, cells are dysregulated in hypertension,[43] demonstrating greater production of pro-inflammatory cytokines including COVID-19-related cytokines (IL-17, IL-7, IL-6, interferon-γ, and TNF-α).[46] More interestingly, hypertension is associated with a particular immunosenescent profile in CD8+ cells,[41,46,47] which are prone to overproduction of cytokines but are less efficient in antiviral defence. These immune mechanisms also contribute significantly to accelerated end-organ damage.[48,49] Taken together, these data indicate accelerated ageing of the immune system in hypertension that may in part explain why hypertension is potentially associated with a more severe course of COVID-19.[47] To test this hypothesis, large-scale observational studies analysing the association between hypertension and COVID-19 infections and outcome with appropriate adjustments, particularly for age, are therefore urgently needed.

How is the renin–angiotensin system linked to lung damage?

The RAS is a major regulator of cardiovascular and renal function including blood pressure control.[50–52] In the classical concept, the principal effector of the RAS is angiotensin II (Ang II) acting on Ang II type 1 receptors (AT_1Rs) and type 2 receptors (AT_2Rs) to mediate its main vascular effects as well as secretion of aldosterone from the adrenals.[50–54] The RAS is, however, a much more complex system with additional components and sites of action that influence not only blood pressure homeostasis and cardiovascular function, but also the function and injury of several organs[50–52,54] including the lung.[55] Ang II is generated by the dipeptidyl-carboxypeptidase ACE, which cleaves Ang I to produce Ang II[56] (*Figure 2A*). An important counter-regulatory pathway within the RAS involves the mono-carboxypeptidase ACE2, angiotensin 1-7 (Ang 1-7), and the Mas receptor (MasR) that counterbalance the classical Ang II–AT_1R axis of the RAS (*Figure 2A*).[51–54] The membrane-anchored ACE2 is a homologue of ACE, but is insensitive to ACEIs,[54,57] and acts as a carboxypeptidase by cleaving single terminal residues from several bioactive peptides. ACE2 thereby converts mainly Ang II to Ang 1-7 and, with much lower efficiency, Ang I to Ang 1-9.[54] Ang 1-9 is also converted to Ang 1-7 by ACE for which it represents a competitive inhibitor, thereby decreasing Ang II levels.[54] The latter effect together with the activation of AT_2R may contribute to protective effects of Ang 1-9.[58] ACE2 induces vasodilation by reducing Ang II effects and by increasing Ang 1-7 synthesis. Ang 1-7 binds to the MasR and thereby exhibits, in addition to nitric oxide release and activation of baroreflex sensitivity, several other effects that protect against tissue injury in the cardiovascular system, kidney,[59] and other organs.[51–53] Indeed, in several lung injury animal models, activation of the Ang II–AT_1R axis has been associated with the severity of lung injury, while the augmentation of ACE2/Ang 1-7/MasR signalling counteracts these detrimental effects (*Figure 2A*).[55] In several acute lung injury models, down-regulation of ACE2 was demonstrated.[55,60] In mouse models of sepsis or acid aspiration, knockout of the gene coding for ACE2 (*ace2*) resulted in a more severe lung injury compared with wild-type mice.[61,62] In contrast, mice deficient for the corresponding genes of ACE (*ace*) or AT_1R (*Ata1a*) showed improved symptoms of acute lung injury.[61] Importantly, increased vascular permeability, one of the hallmarks in the pathogenesis of acute respiratory distress syndrome (ARDS), is particularly pronounced in ACE2-deficient mice.[61] In contrast, the increase in vascular permeability and leakage was significantly attenuated in the lungs of knockout mice for AT_1R (*Ata1a*).[61] Taken together with experimental studies showing beneficial effects of ACEIs[63,64] or ARBs,[65,66] or ACE2 supplementation, these data indicate that loss of ACE2 expression and increased Ang II–AT_1R axis activation promote the disease in lung injury models.[55,60,67]

Due to its terminal carboxypeptidase activity, ACE2 is also involved in the modification of other bioactive peptides[68] including the active metabolite of bradykinin, i.e. des-Arg9 bradykinin (DABK). DABK is a well-known pulmonary inflammatory mediator and ACE2 substrate.[69] A reduction in pulmonary ACE2 activity has recently been associated with activation of the DABK/bradykinin receptor B1 (BKDR1R) axis and more severe acute lung inflammation in a lung injury model in mice in response to endotoxin inhalation.[69] This finding provides further support and implications for the relevance of the network between the kinin–kallikrein system (KKS) and the RAS in several biological and pathological processes.[70] However, it is beyond the scope of this review to further explore the role of ACE2 in the KKS and this network. Moreover, the RAS is also linked to the natriuretic peptide system, another counter-regulatory system that stimulates diuresis, natriuresis, and vasodilation.[54] Natriuretic peptides are inactivated by neprilysin [neutral endopeptidase (NEP)] which represents the therapeutic target for the NEP inhibitor sacubitril.[54] Neprilysin is involved in the cleavage of various biologically active peptides and activates the MasR pathway by generating Ang 1-7 from Ang II (*Figure 2A*). Sacubitril has been developed and approved in combination with an ARB (angiotensin receptor neprilysin inhibitor; ARNI) for treatment of heart failure[71] and is also a potent blood pressure-lowering drug.[54,72]

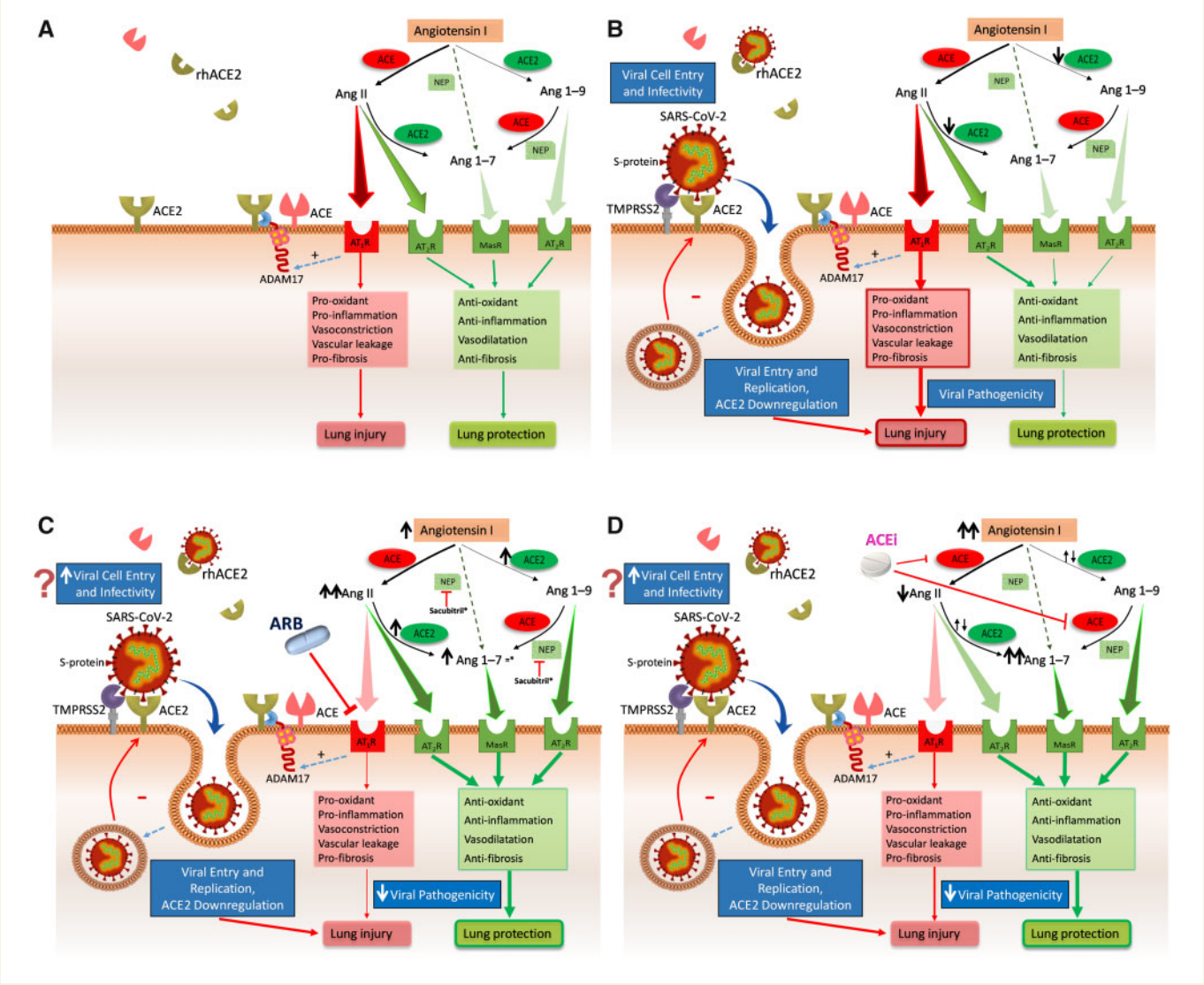


Figure 2 (*A*) Schematic diagram of the RAS in the lung showing the role of ACE2 as a key element in the counter-regulatory axis of the RAS (elements in green; reviewed in Arendse *et al.*[54]). ACE2, a membrane-bound enzyme is cleaved (shedding) by ADAM17 into a soluble form released in the body fluids.[100] ACE2 opposes the harmful effects on lung injury of the Ang II–AT_1R axis (elements in red) by activating MasR and AT_2R signalling. (*B*) ACE2 is expressed in airway epithelial cells including alveolar epithelial type II cells (AECII) in the lung. After infection, SARS-CoV-2 binds through its viral spike protein to host cell membrane-bound ACE2, thereby promoting viral cell entry and subsequent replication. SARS-CoV-2 requires in addition the cellular serine protease TMPRSS2, which will process SARS-CoV-2 by enzymatic cleavage of the spike protein and support cell entry. Importantly, binding of SARS-CoV-2 may lead to down-regulation of ACE2, and thus its own binding receptor required for cell entry. Impairment of ACE2 activity in the lung results in activation of the harmful Ang II–AT_1R axis. This aggravates the viral pathogenicity of SARS-CoV-2, tipping the scale in favour of lung damage. A soluble form of human ACE2 (rhACE2) is currently considered as a therapeutic approach to act as a decoy halting the interaction between SARS-CoV-2 and ACE2 to lessen viral entry.[109] In addition, an inhibitor for TMPRSS2, i.e. camostat mesylate, which is available and approved for other diseases, could be considered for treatment of SARS-CoV-2 by inhibiting cell entry.[6] Pharmacological treatment with ARBs (*C*) or ACEIs (*D*) will modulate several components of the RAS either directly or by affecting feedback loops. Treatment with ARBs protects against lung injury by AT_1R receptor blockade. The corresponding increase in Ang II and Ang I levels will at the same time activate the protective axis and thereby reduce viral pathogenicity. ARBs have been shown to increase ACE2 expression in various tissues, though current evidence for the lungs (particularly in human) is lacking (*Table 1*). Assuming that ARBs can also up-regulate ACE2 in the lung, this will contribute to their protective effect. Protective Ang 1-7, can be also generated by neutral endopeptidase (NEP) or neprilysin. Therefore, the protective effect mediated by Ang1-7 is expected to be lower in response to treatment with ARNIs containing sacubitril. (*D*) Treatment with ACEIs can primarily protect from lung injury by reducing Ang II levels due to the inhibition of Ang I to Ang II conversion. Additional indirect effects supporting the protective axis can contribute to their beneficial effects. An overall effect on lung tissue protection could additionally be promoted by modulation of ACE2, albeit the data supporting this mechanism are scant (*Table 1*). ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; ARNI, angiotensin receptor neprilysin inhibitor; AT_1R, angiotensin II receptor type 1; AT_2R, angiotensin II receptor type 2; MasR, Mas receptor; RAS, renin–angiotensin system; rhACE2, recombinant human ACE2; TMPRSS2, type II transmembrane serine protease. Thicker arrows indicate a predominant pathway or an augmented activation; ↑↑ = up-regulation; ↑↓ = non-consistent effect.

How is the renin–angiotensin system linked to SARS-CoV-2?

The spike protein of SARS-CoV facilitates viral entry into target cells.[6,73] Cell entry into target cells depends on the binding of the spike protein to a cellular receptor, which facilitates viral attachment to the cell surface. In addition, SARS-CoV employs the cellular serine protease TMPRSS2 as a cofactor for cell entry by priming of the spike protein.[6,74,75] Importantly, previous studies demonstrated that ACE2 represents the cellular entry receptor of SARS-CoV by binding with its spike protein to ACE2.[73] It was further shown that the efficiency of ACE2 binding is a key determinant of SARS-CoV transmission.[76,77] Recent studies indicate that SARS-CoV-2 also uses ACE2 for cell entry and also employs TMPRSS2 for priming its spike protein.[5,6] Consequently, ACE2, being the cell entry receptor for SARS-CoV-2, establishes the link between COVID-19 and the RAS (*Figure 2B*). ACE2 is expressed in the lung and several other tissues including the brain, kidney, gastrointestinal tract, and adipose tissue.[50,78] Human ACE2 was previously detected in ciliated airway epithelial cells of human airway tissues derived from nasal or tracheobronchial regions.[79] However, since viral cell entry for SARS-CoV and SARS-CoV-2 depends on both ACE2 and TMPRSS2, co-expression of the two proteins is necessary to acquire infectivity. In this regard, a more recent report demonstrated co-expression of ACE2 and TMPRSS2 in human nasal and respiratory sinuses, bronchial epithelium (in particular glandular epithelium), and alveolar epithelial type II cells (AECII)[80] (Supplementary material online, *Table S1*). In addition, co-expression was also documented in the gastrointestinal system (oesphagus, stomach, ileum, and colon), cardiomyocytes, and variably in blood vessels (endothelial cells and vascular smooth muscle cells) and some other tissues, but the kidney was apparently not analysed.[80] In the kidney, however, at least ACE2 is abundantly expressed, particularly in the brush border of the proximal tubular cells.[81] The potential co-expression of ACE2 and TMPRSS2 in the kidney is indicated in Supplementary material online, *Table S1*. Taken together, ACE2 and TMPRSS2 are co-expressed in most epithelial cells of the human respiratory tract and therefore might support SARS-CoV and SARS-CoV-2 spread in and between individuals.[6,80] Extrarespiratory spread might also be promoted in the gastrointestinal tract and other organs, as has been suggested for SARS-CoV.[82,83] By using novel omic analyses including single-cell transcriptome analysis, co-expression patterns of both ACE2 and TMPRSS2 will reveal further insights into possible transmission and dissemination patterns of SARS-CoV-2.[84,85] Currently, apart from the respiratory system, the role of other organs and tissues for virus transmission and spreading, e.g. in the gastrointestinal tract,[86] is not well defined. Evidence exists that faecal excretion could facilitate faecal–oral transmission for both SARS-CoV and MERS-CoV, but not for SARS-CoV-2.[86] SARS-CoV-2 has been also detected in faeces or rectal swaps of COVID-19 patients, but not in urine samples.[87–89] The viral load of SARS-CoV-2 in respiratory samples has been reported to be much higher as compared with faecal samples and thus may contribute to the preferential transmission of the virus in the respiratory tract.[87] Taken together, the respiratory tract appears as the main target for infectivity and transmission of SARS-CoV-2, and a time course analysis of viral load of SARS-CoV-2 indicated that posterior oropharyngeal saliva samples can be used for initial diagnosis and subsequent viral load monitoring of COVID-19.[89]

Interestingly, Kuba *et al.* demonstrated that both SARS-CoV infection and injection of SARS-CoV spike protein alone into mice reduced ACE2 expression, and this down-regulation was associated with worsening of lung failure[62] (*Figure 2B*). Although it may seem counterintuitive at first that reduction of ACE2 induced by the virus increases lung damage, this is not surprising on closer examination. Indeed, as outlined above, down-regulation of ACE2 was associated with lung injury in several animal models.[55,60,67] In agreement with these observations, Kuba *et al.* demonstrated worsening of lung damage in response to spike protein injection in conjunction with ACE2 suppression.[62] Furthermore, the worsening of lung failure in this setting could be attenuated by treatment with an ARB, clearly demonstrating that the activation of the pulmonary Ang II–AT_1R axis influences the pathogenicity of SARS-CoV infection and that down-regulation of ACE2 is a contributing mechanism,[55,62] as summarized in *Figure 2B*. Loss of pulmonary ACE2 is also thought to mediate acute lung injury induced by influenza viruses in mouse models.[90,91] Collectively, although down-regulation of ACE2 by SARS-CoV-2 has, to our knowledge, not been shown yet, the available data indicate a down-regulation of ACE2 in acute lung injury models and support a protective role for RAS blockade against lung injury in SARS-CoV and by extrapolation also in SARS-CoV-2 infections (*Figure 2B*).

Previous animal studies indicated an age-dependent decrease in pulmonary ACE2 levels, but a recent prospective observational study in mechanically ventilated ARDS patients including neonates, children, and young and older adults (>65 years of age) demonstrated that ACE2 activity (and also ACE activity) in broncho-alveolar lavage fluid was not significantly associated with age.[92] Furthermore, according to a recent report, no supporting evidence was found that genetic variants in the *ACE2* gene, located on the X chromosome, or in corresponding expression quantitative trait loci (eQTLs) could influence the susceptibility to COVID-19 infections.[93] Accordingly, analysis of large-scale bulk transcriptomic data sets of normal lung tissue and single-cell transcriptomic data sets did not reveal significant differences in ACE2 expression between racial groups (Asian vs. Caucasian), age groups (age >60 vs. age <60 years), or gender (male vs. female).[94]

Potential impact of renin–angiotensin system blockers on ACE2 and COVID-19

Treatment with ACEIs and ARBs has a differential impact on several components of the RAS, either directly or by feedback loops (*Figure 2C* and *D*).[54] This also applies to ACE2, although ACE2 is not a direct target of ACEIs because they do not bind and inhibit the active site of ACE2 due to structural differences between ACE and ACE2.[54] However, several reports indicated an up-regulation of ACE2 during treatment with ARBs or ACEIs.[4] In the context of the COVID-19 pandemic, some authors were therefore tempted to speculate that RAS blockers would increase the infectivity of SARS-CoV-2 and severity of the clinical course. Therefore, they suggested to avoid RAS blockers during the COVID-19 pandemic.[95,96] A review of the available literature on this topic revealed that the majority of studies were performed in rat models with a lower number of reports in human cells *in vitro* (summarized in *Table 1*). The few reports that analysed the effects of mineralocorticoid-receptor antagonists (MRAs) on ACE2 in rodents and in human cells in culture do not allow a meaningful assessment (*Table 1*). Taken together, consistent changes of ACE2 mRNA expression and ACE2 protein (activity) in response to RAS blockade were not observed. Secondly, a more consistent up-regulation of ACE2 was observed for ARBs, while the modulation of ACE2 by ACEIs was more variable (*Table 1*). Differences

Table 1 Effect of renin–angiotensin system blockers on ACE2

Study	Drug	Species (strain)	Tissue(s)	Model/disease	Effect on ACE2 (mRNA/activity or protein)
Angiotensin receptor blockers					
Ishiyama *et al.*[110]	Losartan, olmesartan	Rat (Lewis)	Heart	Myocardial infarction	↑/NA
Ferrario *et al.*[111]	Losartan	Rat (Lewis)	Heart	Normotension	↑/↑
Ferrario *et al.*[112]	Losartan	Rat (Lewis)	Kidney	Normotension	↔/↑
Igase *et al.*[113]	Olmesartan	Rat (SHR)	Aorta	Hypertension	↑/↑
Karram *et al.*[114]	Eprosartan	Rat (Wistar)	Heart	Heart failure	NA/↑
Agata *et al.*[115]	Olmesartan	Rat (SHRSP)	Heart	Hypertension	↑/NA
Agata *et al.*[115]	Olmesartan	Rat (SHRSP)	Kidney	Hypertension	NA/↑*
Jessup *et al.*[116]	Losartan	Rat (Ren 2 transgenic)	Heart, kidney	Hypertension	↑/↑
Ocaranza *et al.*[117]	Enalapril	Rat (Sprague–Dawley)	Heart/plasma	Myocardial infarction	↑/↑
Whaley-Connell *et al.*[118]	Valsartan	Rat (Ren 2 transgenic)	Kidney	Hypertension	↑/NA
Takeda *et al.*[119]	Candesartan	Rat (Dahl)	Heart	Salt-sensitive hypertension	↑/↑*
Gilliam-Davis *et al.*[120]	L-158,809	Rat (Fischer344)	Brain (dorsomedial medulla)	Normotension	↑/NA
Sukumaran *et al.*[121]	Telmisartan	Rat (Lewis)	Heart	Autoimmune myocarditis	NA/↑*
Sukumaran *et al.*[122]	Olmesartan	Rat (Lewis)	Heart	Dilated cardiomyopathy	↑/↑*
Vuille-dit-Bille *et al.*[102]	Not indicated	Human	Duodenal biopsies	Not indicated	↔/NA
Lezama-Martinez *et al.*[123]	Losartan	Rat (SHR)	Aorta	Hypertension	↓/NA
Angiotensin-converting enzyme inhibitors					
Burrell *et al.*[124]	Ramipril	Rat (Sprague–Dawley)	Heart	Myocardial infarction	↔/↔
Ferrario *et al.*[111]	Lisinopril	Rat (Lewis)	Heart	Normotension	↑/↔
Ferrario *et al.*[112]	Lisinopril	Rat (Lewis)	Renal cortex	Normotension	↔/↑
Jessup *et al.*[116]	Lisinopril	Rat (Ren 2 transgenic)	Heart, kidney	Hypertension	↑/↑
Hamming *et al.*[125]	Lisinopril	Rat (Wistar)	Kidney	Normotension (plus high NaCl diet)	↓/↔
Vuille-dit-Bille *et al.*[102]	Not reported	Human	Duodenal biopsies	Not reported	↑/NA
Lezama-Martinez *et al.*[123]	Captopril	Rat (SHR)	Aorta	Hypertension	↓/NA
Mineralocorticoid receptor antagonists					
Keider *et al.*[126]	Spironolactone	Human	Cultured monocyte-derived macrophages	Heart failure	↑/↑
	Eplerenone	Mouse (Balb/C)	Cultured peritoneal macrophages	Normal	↑/↑
			Heart		↑/↑
			Kidney		↑/↔
Karram *et al.*[114]	Spironolactone	Rat	Heart	Heart failure	NA/↑
Takeda *et al.*[119]	Eplerenone	Rat (Dahl)	Heart	Salt-sensitive hypertension	↔/↔
Stoll *et al.*[127]	Spironolactone	Human	Cultured mesangial cells	–	NA/↔

NA, not analysed; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; ↑ = increased; ↓ = decreased; * indicates ACE2 protein level; ↔ = no effect.

between studies may depend on the animal strains used, different pathophysiological conditions, e.g. normotension vs. hypertension, and the organs investigated. Furthermore, the type and dosage of drugs used may play a role. Studies that investigate the effect of RAS blockade on ACE2 in humans are scarce. Furuhashi *et al.* showed a significant increase in the urinary excretion of ACE2 in hypertensive patients after treatment with an ARB for >1 year when compared with other antihypertensive agents including the ACEI enalapril.[97] A similar observation was reported in patients with diabetic nephropathy after chronic treatment with an ARB for 24 weeks.[98] In another study, however, the use of ARBs or ACEIs did not affect urinary ACE2 levels in patients with diabetes.[99]

Taken together, these studies are not conclusive due to the complexity of measuring the circulating and urine levels of ACE2, since ACE2 is mainly a membrane-anchored enzyme. Physiologically, soluble ACE2 is generated by cleavage (shedding) of membrane-bound ACE2 by the disintegrin and metalloprotease 17 (ADAM17)[100] (*Figure 2A*). This mechanism also underlies the occurrence of ACE2 in the urine and other body fluids. Since ADAM17 is up-regulated by AT_1R signalling (*Figure 2A*), urinary ACE2 originating by shedding membrane-bound ACE2 from the proximal tubular apical brush border might increase in response to activation of the renal RAS.[101] Thus, it is expected that this effect will be blunted by ARBs. It therefore appears very difficult to predict the

direction of ACE2 regulation in the kidney by urine analysis. Hence, the response to pharmacological RAS blockade by analysing ACE2 in the urine is highly complex due to the difference between the regulation of membrane-bound ACE2 and soluble ACE2, and the interplay of pathophysiological conditions and pharmacological interventions precluding meaningful conclusions. In this regard, one study performing analysis of ACE2 expression in biopsies obtained during gastroduodenoscopy and ileocolonoscopy is worth mentioning.[102] First, the authors demonstrated moderate expression of both ACE2 mRNA and protein in the brush border of small intestine enterocytes (duodenum and terminal ileum) and only low expression in the colon. Secondly, in duodenal samples, ACE2 was significantly (1.9-fold) up-regulated in subjects treated with an ACEI but not in subjects treated with an ARB as compared with untreated controls.[102] In contrast, and importantly, an up-regulation of ACE2 in the lung tissue in response to RAS blockade, particularly in humans, has not been reported.

Summary

The initially reported observational data suggesting an association between hypertension and COVID-19 are likely to be confounded by age and other co-morbidities. In LRTIs, diabetes and heart failure are associated with a complicated course, but hypertension *per se* is not. In contrast, low blood pressure values[11] and blood pressure drops within the first 24 h after hospital admission, as recently shown for CAP,[103] may represent risk factors for adverse outcome in COVID-19. This clearly highlights the need to closely monitor severely ill COVID-19 patients with haemodynamic instability and to withhold and adapt blood pressure-lowering therapies according to guidelines.[104] COVID-19 patients with hypotension and particularly critically ill patients are highly susceptible to acute kidney injury (AKI).[3,105] Similarly, acute cardiac injury as evidenced by increased high-sensitivity troponin levels has been reported in COVID-19 patients and is possibly associated with the high inflammatory burden.[38,106,107] Regardless of the as yet unsolved question of whether the heart or kidneys are target organs for direct viral entry and replication of SARS-COV-2, the development of AKI and cardiac damage in patients with severe COVID-19 disease will clearly influence therapeutic strategies including the use of RAS blockers. This applies in particular to the critical use of these agents in AKI patients with significantly reduced renal function.[108] In contrast to these severely ill patients, because of concerns during a COVID-19 pandemic, RAS blockers should not be discontinued or withheld in stable patients with hypertension and associated diseases, e.g. heart failure, chronic kidney diseases, and diabetes, when indicated.[21,22] Hence, there is currently no evidence to support the concept that use of ACEIs or ARBs could be harmful by increasing SARS-CoV-2 infectivity (*Figure 3*). Impairing the backbone of pharmacotherapy in hypertension or heart failure by withholding or discontinuing RAS blockers in stable patients will have a significant impact on cardiovascular morbidity and mortality given the high prevalence of the diseases and the proven benefit of therapy.[21,22,71] In addition, there is ample experimental evidence that activation of the Ang II–AT_1R axis promotes lung injury, while increases of ACE2 protect against lung injury (*Figure 2C* and *D*). The review of the available clinical studies in patients with LRTI and CAP support a beneficial effect of RAS inhibition in other infections. The potential of protecting lung injury by supplementing ACE2 has led to a clinical proof-of-concept study using recombinant human soluble ACE2 (rhACE2) in patients with COVID-19 (Clinicaltrials.gov #NCT04287686, Figure 2B). It remains to be seen whether this approach supplementing the protective axis of the RAS will be efficacious against COVID-19. In the meantime, however, the use of RAS blockers that inhibit the damaging (Ang II–AT_1R) arm of the RAS cascade in the lung appears reasonable, rather than harmful to us.

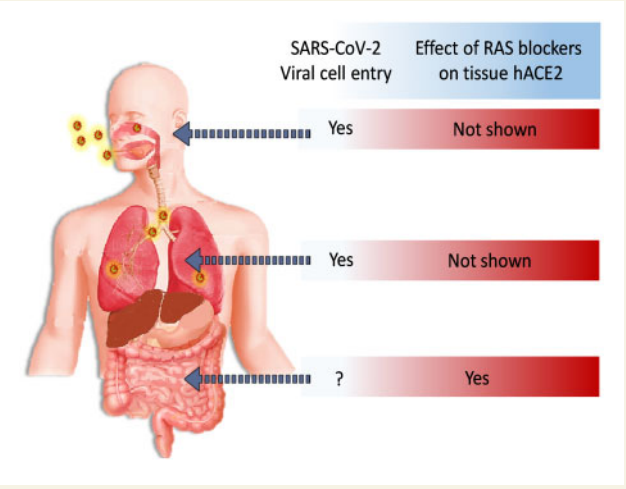


Figure 3 The impact of RAS blockers on human ACE2 (hACE2) expression and SARS-CoV-2 viral cell entry is shown. Currently, no studies have reported on the effects of RAS blockers on tissue ACE2 activity in the upper or the lower respiratory tract. ACE2 is expressed in the oropharynx, ciliated upper airway epithelial cells. and in alveolar epithelial cells type II in the lung. Expression of ACE2 in the oropharynx may facilitate viral cell entry. ACE2 is also abundantly expressed in the gastrointestinal tract, particularly in the brush border membrane of human small intestine enterocytes. However, SARS-CoV-2 infectivity in the gastrointestinal tract is still poorly defined, while SARS-CoV-2 has been detected in rectal swabs or faeces of COVID-19 patients, but not yet in urine samples.

Supplementary material

Supplementary material is available at *Cardiovascular Reseach* online.

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References

1. http://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic. (30 March 2020)
2. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020;doi: 10.1016/S1473-3099(20)30120-1.
3. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;**395**:1054–1062.
4. Soler MJ, Barrios C, Oliva R, Batlle D. Pharmacologic modulation of ACE2 expression. *Curr Hypertens Rep* 2008;**10**:410–414.
5. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**:270–273.
6. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Muller MA, Drosten C, Pohlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;doi: 10.1016/j.cell.2020.02.052.
7. https://www.eshonline.org/spotlights/esh-stabtement-on-covid-19/. (30 March 2020).
8. https://www.escardio.org/Councils/Council-on-Hypertension-(CHT)/News/position-statement-of-the-esc-council-on-hypertension-on-ace-inhibitors-and-ang. (30 March 2020).
9. https://ish-world.com/news/a/A-statement-from-the-International-Society-of-Hypertension-on-COVID-19/. (30 March 2020**).**
10. https://www.whleague.org/index.php/2014-07-09-22-47-11/covid-19-hypertension-guidance. (30 March 2020).
11. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M, Ortqvist A, Schaberg T, Torres A, van der Heijden G, Read R, Verheij TJ. Guidelines for the management of adult lower respiratory tract infections—full version. *Clin Microbiol Infect* 2011;**17** Suppl 6:E1–E59.
12. Gutierrez F, Masia M, Mirete C, Soldan B, Rodriguez JC, Padilla S, Hernandez I, Royo G, Martin-Hidalgo A. The influence of age and gender on the population-based incidence of community-acquired pneumonia caused by different microbial pathogens. *J Infect* 2006;**53**:166–174.
13. Koivula I, Sten M, Makela PH. Risk factors for pneumonia in the elderly. *Am J Med* 1994;**96**:313–320.
14. Corrales-Medina VF, Musher DM, Wells GA, Chirinos JA, Chen L, Fine MJ. Cardiac complications in patients with community-acquired pneumonia: incidence, timing, risk factors, and association with short-term mortality. *Circulation* 2012;**125**: 773–781.
15. Choi J, Jang J, An Y, Park SK. Blood pressure and the risk of death from non-cardiovascular diseases: a population-based cohort study of Korean adults. *J Prev Med Public Health* 2018;**51**:298–309.
16. Cilli A, Erdem H, Karakurt Z, Turkan H, Yazicioglu-Mocin O, Adiguzel N, Gungor G, Bilge U, Tasci C, Yilmaz G, Oncul O, Dogan-Celik A, Erdemli O, Oztoprak N, Samur AA, Tomak Y, Inan A, Karaboga B, Tok D, Temur S, Oksuz H, Senturk O, Buyukkocak U, Yilmaz-Karadag F, Ozcengiz D, Karakas A, Savasci U, Ozgen-Alpaydin A, Kilic E, Elaldi N, Bilgic H. Community-acquired pneumonia in patients with chronic obstructive pulmonary disease requiring admission to the intensive care unit: risk factors for mortality. *J Crit Care* 2013;**28**:975–979.
17. Caldeira D, Alarcao J, Vaz-Carneiro A, Costa J. Risk of pneumonia associated with use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers: systematic review and meta-analysis. *BMJ* 2012;**345**:e4260.
18. Shah S, McArthur E, Farag A, Nartey M, Fleet JL, Knoll GA, Kim SJ, Garg AX, Jain AK. Risk of hospitalization for community acquired pneumonia with renin–angiotensin blockade in elderly patients: a population-based study. *PLoS One* 2014;**9**: e110165.
19. Wu A, Good C, Downs JR, Fine MJ, Pugh MJ, Anzueto A, Mortensen EM. The association of cardioprotective medications with pneumonia-related outcomes. *PLoS One* 2014;**9**:e85797.
20. Kang JH, Kao LT, Lin HC, Wang TJ, Yang TY. Do outpatient statins and ACEIs/ARBs have synergistic effects in reducing the risk of pneumonia? A population-based case–control study. *PLoS One* 2018;**13**:e0199981.
21. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen SE, Kreutz R, Laurent S, Lip GYH, McManus R, Narkiewicz K, Ruschitzka F, Schmieder RE, Shlyakhto E, Tsioufis C, Aboyans V, Desormais I. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of *Hypertension. J Hypertens* 2018;**36**:1953–2041.
22. Whelton PK, Carey RM, Aronow WS,Casey DE Jr, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith SC Jr Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams KA Sr Williamson JD, Wright JTJr. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2018;**71**:e127–e248.
23. Soto M, Bang SI, McCombs J, Rodgers KE. Renin angiotensin system-modifying therapies are associated with improved pulmonary health. *Clin Diabetes Endocrinol* 2017; **3**:6.
24. Henry C, Zaizafoun M, Stock E, Ghamande S, Arroliga AC, White HD. Impact of angiotensin-converting enzyme inhibitors and statins on viral pneumonia. *Proc (Bayl Univ Med Cent)* 2018;**31**:419–423.
25. Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, Alexander L, Estep K, Hassen Abate K, Akinyemiju TF, Ali R, Alvis-Guzman N, Azzopardi P, Banerjee A, Barnighausen T, Basu A, Bekele T, Bennett DA, Biadgilign S, Catala-Lopez F, Feigin VL, Fernandes JC, Fischer F, Gebru AA, Gona P, Gupta R, Hankey GJ, Jonas JB, Judd SE, Khang YH, Khosravi A, Kim YJ, Kimokoti RW, Kokubo Y, Kolte D, Lopez A, Lotufo PA, Malekzadeh R, Melaku YA, Mensah GA, Misganaw A, Mokdad AH, Moran AE, Nawaz H, Neal B, Ngalesoni FN, Ohkubo T, Pourmalek F, Rafay A, Rai RK, Rojas-Rueda D, Sampson UK, Santos IS, Sawhney M, Schutte AE, Sepanlou SG, Shifa GT, Shiue I, Tedla BA, Thrift AG, Tonelli M, Truelsen T, Tsilimparis N, Ukwaja KN, Uthman OA, Vasankari T, Venketasubramanian N, Vlassov VV, Vos T, Westerman R, Yan LL, Yano Y, Yonemoto N, Zaki ME, Murray CJ. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990–2015. *JAMA* 2017;**317**:165–182.
26. Wang Z, Chen Z, Zhang L, Wang X, Hao G, Zhang Z, Shao L, Tian Y, Dong Y, Zheng C, Wang J, Zhu M, Weintraub WS, Gao R. Status of hypertension in China: results from the China Hypertension Survey, 2012–2015. *Circulation* 2018;**137**: 2344–2356.
27. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2020;**41**:145–151.
28. Williams B, Mancia G, Spiering W, Rosei EA, Azizi M, Burnier M, Clement D, Coca A, De Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen S, Kreutz R, Laurent S, Lip GYH, McManus R, Narkiewicz K, Ruschitzka F, Schmieder R, Shlyakhto E, Tsioufis K, Aboyans V, Desormais I. 2018 Practice Guidelines for the management of arterial hypertension of the European Society of Hypertension and the European Society of Cardiology: ESH/ESC Task Force for the Management of Arterial Hypertension. *J Hypertens* 2018;**36**: 2284–2309.
29. Fauci AS, Lane HC, Redfield RR. Covid-19—navigating the uncharted. *N Engl J Med* 2020;**382**:1268–1269.
30. Grasselli G, Pesenti A, Cecconi M. Critical care utilization for the COVID-19 outbreak in Lombardy, Italy: early experience and forecast during an emergency response. *JAMA* 2020;doi: 10.1001/jama.2020.4031.
31. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *Lancet* 2020;doi: 10.1016/S0140-6736(20)30627-9.
32. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA* 2020;doi: 10.1001/jama.2020.4683.
33. https://www.epicentro.iss.it/coronavirus/sars-cov-2-decessi-italia. (30 M**arch** 2020**).**
34. Tocci G, Nati G, Cricelli C, Parretti D, Lapi F, Ferrucci A, Borghi C, Volpe M. Prevalence and control of hypertension in the general practice in Italy: updated analysis of a large database. *J Hum Hypertens* 2017;**31**:258–262.
35. Fisher D, Heymann D. Q&A: The novel coronavirus outbreak causing COVID-19. *BMC Med* 2020;**18**:57.
36. Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarity in case fatality rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. *J Infect Dev Ctries* 2020;**14**: 125–128.
37. Drummond G, Vinh A, Guzik T, Sobey CG. Immune mechanisms of hypertension. *Nat Rev Immunol* 2019;**19**:517–532.
38. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, *China. Lancet* 2020;**395**:497–506.
39. Carnevale D, Wenzel P. Mechanical stretch on endothelial cells interconnects innate and adaptive immune response in hypertension. *Cardiovasc Res* 2018;**114**: 1432–1434.
40. Loperena R, Van Beusecum JP, Itani HA, Engel N, Laroumanie F, Xiao L, Elijovich F, Laffer CL, Gnecco JS, Noonan J, Maffia P, Jasiewicz-Honkisz B, Czesnikiewicz-Guzik M, Mikolajczyk T, Sliwa T, Dikalov S, Weyand CM, Guzik TJ, Harrison DG. Hypertension and increased endothelial mechanical stretch promote monocyte differentiation and activation: roles of STAT3, interleukin 6 and hydrogen peroxide. *Cardiovasc Res* 2018;**114**:1547–1563.
41. Czesnikiewicz-Guzik M, Osmenda G, Siedlinski M, Nosalski R, Pelka P, Nowakowski D, Wilk G, Mikolajczyk TP, Schramm-Luc A, Furtak A, Matusik P, Koziol J, Drozdz

M, Munoz-Aguilera E, Tomaszewski M, Evangelou E, Caulfield M, Grodzicki T, D'Aiuto F, Guzik TJ. Causal association between periodontitis and hypertension: evidence from Mendelian randomization and a randomized controlled trial of non-surgical periodontal therapy. *Eur Heart J* 2019;**40**:3459–3470.
42. China NHCotPsRo. *Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment*, 7th ed. http://kjfymeetingchinaorg/msite/news/show/cn/3337html 2020.
43. Perrotta M, Lori A, Carnevale L, Fardella S, Cifelli G, Iacobucci R, Mastroiacovo F, Iodice D, Pallante F, Storto M, Lembo G, Carnevale D. Deoxycorticosterone acetate-salt hypertension activates placental growth factor in the spleen to couple sympathetic drive and immune system activation. *Cardiovasc Res* 2018;**114**: 456–467.
44. Chou CH, Hung CS, Liao CW, Wei LH, Chen CW, Shun CT, Wen WF, Wan CH, Wu XM, Chang YY, Wu VC, Wu KD, Lin YH, TAIPAI Study Group. IL-6 trans-signalling contributes to aldosterone-induced cardiac fibrosis. *Cardiovasc Res* 2018; **114**:690–702.
45. Siedlinski M, Jozefczuk E, Xu X, Teumer A, Evangelou E, Schnabel RB, Welsh P, Maffia P, Erdmann J, Tomaszewski M, Caulfield MJ, Sattar N, Holmes MV, Guzik TJ. White blood cells and blood pressure: a Mendelian randomization study. *Circulation* 2020;doi: 10.1161/CIRCULATIONAHA.119.045102.
46. Itani HAMcMaster WG Jr, Saleh MA, Nazarewicz RR, Mikolajczyk TP, Kaszuba AM, Konior A, Prejbisz A, Januszewicz A, Norlander AE, Chen W, Bonami RH, Marshall AF, Poffenberger G, Weyand CM, Madhur MS, Moore DJ, Harrison DG, Guzik TJ. Activation of human T cells in hypertension: studies of humanized mice and hypertensive humans. *Hypertension* 2016;**68**:123–132.
47. Youn JC, Yu HT, Lim BJ, Koh MJ, Lee J, Chang DY, Choi YS, Lee SH, Kang SM, Jang Y, Yoo OJ, Shin EC, Park S. Immunosenescent CD8+ T cells and C-X-C chemokine receptor type 3 chemokines are increased in human hypertension. *Hypertension* 2013;**62**:126–133.
48. Ketelhuth DFJ. The immunometabolic role of indoleamine 2,3-dioxygenase in atherosclerotic cardiovascular disease: immune homeostatic mechanisms in the artery wall. *Cardiovasc Res* 2019;**115**:1408–1415.
49. Ketelhuth DFJ, Lutgens E, Back M, Binder CJ, Van den Bossche J, Daniel C, Dumitriu IE, Hoefer I, Libby P, O'Neill L, Weber C, Evans PC. Immunometabolism and atherosclerosis: perspectives and clinical significance: a position paper from the Working Group on Atherosclerosis and Vascular Biology of the European Society of Cardiology. *Cardiovasc Res* 2019;**115**:1385–1392.
50. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin–angiotensin systems. *Physiol Rev* 2006;**86**:747–803.
51. Romero CA, Orias M, Weir MR. Novel RAAS agonists and antagonists: clinical applications and controversies. *Nat Rev Endocrinol* 2015;**11**:242–252.
52. Ferrario CM, Mullick AE. Renin angiotensin aldosterone inhibition in the treatment of cardiovascular disease. *Pharmacol Res* 2017;**125**:57–71.
53. Oparil S, Schmieder RE. New approaches in the treatment of hypertension. *Circ Res* 2015;**116**:1074–1095.
54. Arendse LB, Danser AHJ, Poglitsch M, Touyz RM, Burnett JC Jr, Llorens-Cortes C, Ehlers MR, Sturrock ED. Novel therapeutic approaches targeting the renin–angiotensin system and associated peptides in hypertension and heart failure. *Pharmacol Rev* 2019;**71**:539–570.
55. Imai Y, Kuba K, Penninger JM. The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. *Exp Physiol* 2008;**93**:543–548.
56. Corvol P, Williams TA, Soubrier F. Peptidyl dipeptidase A: angiotensin I-converting enzyme. *Methods Enzymol* 1995;**248**:283–305.
57. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000;**275**:33238–33243.
58. Fattah C, Nather K, McCarroll CS, Hortigon-Vinagre MP, Zamora V, Flores-Munoz M, McArthur L, Zentilin L, Giacca M, Touyz RM, Smith GL, Loughrey CM, Nicklin SA. Gene therapy with angiotensin-(1-9) preserves left ventricular systolic function after myocardial infarction. *J Am Coll Cardiol* 2016;**68**:2652–2666.
59. Ingelfinger JR. Angiotensin-converting enzyme 2: implications for blood pressure and kidney disease. *Curr Opin Nephrol Hypertens* 2009;**18**:79–84.
60. Ye R, Liu Z. ACE2 exhibits protective effects against LPS-induced acute lung injury in mice by inhibiting the LPS–TLR4 pathway. *Exp Mol Pathol* 2020;**113**: 104350.
61. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;**436**:112–116.
62. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11**:875–879.
63. Li Y, Zeng Z, Li Y, Huang W, Zhou M, Zhang X, Jiang W. Angiotensin-converting enzyme inhibition attenuates lipopolysaccharide-induced lung injury by regulating the balance between angiotensin-converting enzyme and angiotensin-converting enzyme 2 and inhibiting mitogen-activated protein kinase activation. *Shock* 2015;**43**: 395–404.
64. Boskabadi J, Askari VR, Hosseini M, Boskabady MH. Immunomodulatory properties of captopril, an ACE inhibitor, on LPS-induced lung inflammation and fibrosis as well as oxidative stress. *Inflammopharmacology* 2019;**27**:639–647.
65. Liu L, Qiu HB, Yang Y, Wang L, Ding HM, Li HP. Losartan, an antagonist of AT1 receptor for angiotensin II, attenuates lipopolysaccharide-induced acute lung injury in rat. *Arch Biochem Biophys* 2009;**481**:131–136.
66. Deng W, Deng Y, Deng J, Wang DX, Zhang T. Losartan attenuated lipopolysaccharide-induced lung injury by suppression of lectin-like oxidized low-density lipoprotein receptor-1. *Int J Clin Exp Pathol* 2015;**8**:15670–15676.
67. Tan WSD, Liao W, Zhou S, Mei D, Wong WF. Targeting the renin–angiotensin system as novel therapeutic strategy for pulmonary diseases. *Curr Opin Pharmacol* 2018; **40**:9–17.
68. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem* 2002;**277**:14838–14843.
69. Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, McCray PB Jr, Chappell M, Hackam DJ, Jia H. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg(9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. *Am J Physiol Lung Cell Mol Physiol* 2018;**314**:L17–Ll31.
70. Stoka V, Turk V. A structural network associated with the kallikrein–kinin and renin–angiotensin systems. *Biol Chem* 2010;**391**:443–454.
71. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;**37**:2129–2200.
72. Ruilope LM, Dukat A, Bohm M, Lacourciere Y, Gong J, Lefkowitz MP. Blood-pressure reduction with LCZ696, a novel dual-acting inhibitor of the angiotensin II receptor and neprilysin: a randomised, double-blind, placebo-controlled, active comparator study. *Lancet* 2010;**375**:1255–1266.
73. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003;**426**:450–454.
74. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *J Virol* 2010;**84**:12658–12664.
75. Glowacka I, Bertram S, Muller MA, Allen P, Soilleux E, Pfefferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, Niemeyer D, Schneider H, Drosten C, Pohlmann S. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol* 2011;**85**:4122–4134.
76. Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, Wong S-K, Huang I-C, Xu K, Vasilieva N, Murakami A, He Y, Marasco WA, Guan Y, Choe H, Farzan M. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. *The EMBO Journal* 2005;**24**:1634–1643.
77. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 2005;**309**:1864–1868.
78. Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, Campagnole-Santos MJ. The ACE2/angiotensin-(1-7)/MAS axis of the renin–angiotensin system: focus on angiotensin-(1-7). *Physiol Rev* 2018;**98**:505–553.
79. Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. *J Virol* 2005;**79**: 15511–15524.
80. Bertram S, Heurich A, Lavender H, Gierer S, Danisch S, Perin P, Lucas JM, Nelson PS, Pohlmann S, Soilleux EJ. Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts. *PLoS One* 2012;**7**:e35876.
81. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004;**203**:631–637.
82. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, Wang H, Shen H, Qiu L, Li Z, Geng J, Cai J, Han H, Li X, Kang W, Weng D, Liang P, Jiang S. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol* 2004;**203**:622–630.
83. Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, Zou W, Zhan J, Wang S, Xie Z, Zhuang H, Wu B, Zhong H, Shao H, Fang W, Gao D, Pei F, Li X, He Z, Xu D, Shi X, Anderson VM, Leong AS. Multiple organ infection and the pathogenesis of SARS. *J Exp Med* 2005;**202**:415–424.
84. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, Fernandez R, Akbarpour M, Chen CI, Ren Z, Verma R, Abdala-Valencia H, Nam K, Chi M, Han S, Gonzalez-Gonzalez FJ, Soberanes S, Watanabe S, Williams KJN, Flozak AS, Nicholson TT, Morgan VK, Winter DR, Hinchcliff M, Hrusch CL, Guzy RD, Bonham CA, Sperling AI, Bag R, Hamanaka RB, Mutlu GM, Yeldandi AV,

Marshall SA, Shilatifard A, Amaral LAN, Perlman H, Sznajder JI, Argento AC, Gillespie CT, Dematte J, Jain M, Singer BD, Ridge KM, Lam AP, Bharat A, Bhorade SM, Gottardi CJ, Budinger GRS, Misharin AV. Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. *Am J Respir Crit Care Med* 2019;**199**:1517–1536.
85. Chu SG, Poli De Frias S, Sakairi Y, Kelly RS, Chase R, Konishi K, Blau A, Tsai E, Tsoyi K, Padera RF, Sholl LM, Goldberg HJ, Mallidi HR, Camp PC, El-Chemaly SY, Perrella MA, Choi AMK, Washko GR, Raby BA, Rosas IO. Biobanking and cryopreservation of human lung explants for omic analysis. *Eur Respir J* 2020;**55**: 1801635.
86. Yeo C, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal–oral transmission of SARS-CoV-2 possible? *Lancet Gastroenterol Hepatol* 2020;**5**:335–337.
87. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 2020;**20**:411–412.
88. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* 2020;doi: 10.1001/jama.2020.3786.
89. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CC, Cai JP, Chan JM, Chik TS, Lau DP, Choi CY, Chen LL, Chan WM, Chan KH, Ip JD, Ng AC, Poon RW, Luo CT, Cheng VC, Chan JF, Hung IF, Chen Z, Chen H, Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020; doi: 10.1016/S1473-3099(20)30196-1.
90. Yang P, Gu H, Zhao Z, Wang W, Cao B, Lai C, Yang X, Zhang L, Duan Y, Zhang S, Chen W, Zhen W, Cai M, Penninger JM, Jiang C, Wang X. Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci Rep* 2014;**4**:7027.
91. Zou Z, Yan Y, Shu Y, Gao R, Sun Y, Li X, Ju X, Liang Z, Liu Q, Zhao Y, Guo F, Bai T, Han Z, Zhu J, Zhou H, Huang F, Li C, Lu H, Li N, Li D, Jin N, Penninger JM, Jiang C. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat Commun* 2014;**5**:3594.
92. Schouten LR, van Kaam AH, Kohse F, Veltkamp F, Bos LD, de Beer FM, van Hooijdonk RT, Horn J, Straat M, Witteveen E, Glas GJ, Wieske L, van Vught LA, Wiewel MA, Ingelse SA, Cortjens B, van Woensel JB, Bos AP, Walther T, Schultz MJ, Wosten-van Asperen RM. Age-dependent differences in pulmonary host responses in ARDS: a prospective observational cohort study. *Ann Intensive Care* 2019;**9**:55.
93. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, Wen F, Huang X, Ning G, Wang W. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. *Cell Discov* 2020;**6**:11.
94. Cai G. Bulk and single-cell transcriptomics identify tobacco-use disparity in lung gene expression of ACE2, the receptor of 2019-*nCov*. *medRxiv* **2020**: 2020.2002.2005.20020107.
95. 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.
96. Esler M, Esler D. Can angiotensin receptor-blocking drugs perhaps be harmful in the COVID-19 pandemic? *J Hypertens* 2020;**38**:781–782.
97. Furuhashi M, Moniwa N, Mita T, Fuseya T, Ishimura S, Ohno K, Shibata S, Tanaka M, Watanabe Y, Akasaka H, Ohnishi H, Yoshida H, Takizawa H, Saitoh S, Ura N, Shimamoto K, Miura T. Urinary angiotensin-converting enzyme 2 in hypertensive patients may be increased by olmesartan, an angiotensin II receptor blocker. *Am J Hypertens* 2015;**28**:15–21.
98. Abe M, Oikawa O, Okada K, Soma M. Urinary angiotensin-converting enzyme 2 increases in diabetic nephropathy by angiotensin II type 1 receptor blocker olmesartan. *J Renin Angiotensin Aldosterone Syst* 2015;**16**:159–164.
99. Mariana CP, Ramona PA, Ioana BC, Diana M, Claudia RC, Stefan VD, Maria KI. Urinary angiotensin converting enzyme 2 is strongly related to urinary nephrin in type 2 diabetes patients. *Int Urol Nephrol* 2016;**48**:1491–1497.
100. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *J Virol* 2014;**88**:1293–1307.
101. Danser AHJ, Epstein M, Batlle D. Renin–angiotensin system blockers and the COVID-19 pandemic: at present there is no evidence to abandon renin–angiotensin system blockers. *Hypertension* 2020;doi: 10.1161/HYPERTENSIONAHA.120.15082.
102. Vuille-dit-Bille RN, Camargo SM, Emmenegger L, Sasse T, Kummer E, Jando J, Hamie QM, Meier CF, Hunziker S, Forras-Kaufmann Z, Kuyumcu S, Fox M, Schwizer W, Fried M, Lindenmeyer M, Gotze O, Verrey F. Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. *Amino Acids* 2015;**47**:693–705.
103. Schulte-Hubbert B, Meiswinkel N, Kutschan U, Kolditz M. Prognostic value of blood pressure drops during the first 24 h after hospital admission for risk stratification of community-acquired pneumonia: a retrospective cohort study. *Infection* 2020;**48**: 267–274.
104. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med* 2017;**43**:304–377.
105. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *JAMA* 2020;doi: 10.1001/jama.2020.4326.
106. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiology* 2020;doi: 10.1001/jamacardio.2020.1286.
107. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol* 2020;doi: 10.1001/jamacardio.2020.1017.
108. Tomson C, Tomlinson LA. Stopping RAS inhibitors to minimize AKI: more harm than good? *Clin J Am Soc Nephrol* 2019;**14**:617–619.
109. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 2020;**46**:586–590.
110. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* 2004;**43**:970–976.
111. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 2005; **111**:2605–2610.
112. Ferrario CM, Jessup J, Gallagher PE, Averill DB, Brosnihan KB, Ann Tallant E, Smith RD, Chappell MC. Effects of renin–angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors. *Kidney Int* 2005;**68**:2189–2196.
113. Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM. Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1-7) expression in the aorta of spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2005;**289**:H1013–H1019.
114. Karram T, Abbasi A, Keidar S, Golomb E, Hochberg I, Winaver J, Hoffman A, Abassi Z. Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure. *Am J Physiol Heart Circ Physiol* 2005;**289**:H1351–H1358.
115. Agata J, Ura N, Yoshida H, Shinshi Y, Sasaki H, Hyakkoku M, Taniguchi S, Shimamoto K. Olmesartan is an angiotensin II receptor blocker with an inhibitory effect on angiotensin-converting enzyme. *Hypertens Res* 2006;**29**:865–874.
116. Jessup JA, Gallagher PE, Averill DB, Brosnihan KB, Tallant EA, Chappell MC, Ferrario CM. Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. *Am J Physiol Heart Circ Physiol* 2006;**291**: H2166–2172.
117. Ocaranza MP, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, Roman M, Ramirez C, Copaja M, Diaz-Araya G, Castro P, Lavandero S. Enalapril attenuates downregulation of angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* 2006;**48**:572–578.
118. Whaley-Connell AT, Chowdhury NA, Hayden MR, Stump CS, Habibi J, Wiedmeyer CE, Gallagher PE, Tallant EA, Cooper SA, Link CD, Ferrario C, Sowers JR. Oxidative stress and glomerular filtration barrier injury: role of the renin–angiotensin system in the Ren2 transgenic rat. *Am J Physiol Renal Physiol* 2006;**291**: F1308–F1314.
119. Takeda Y, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in Dahl salt-sensitive hypertensive rats. *Am J Hypertens* 2007;**20**:1119–1124.
120. Gilliam-Davis S, Gallagher PE, Payne VS, Kasper SO, Tommasi EN, Westwood BM, Robbins ME, Chappell MC, Diz DI. Long-term systemic angiotensin II type 1 receptor blockade regulates mRNA expression of dorsomedial medulla renin–angiotensin system components. *Physiol Genomics* 2011;**43**:829–835.
121. Sukumaran V, Veeraveedu PT, Gurusamy N, Yamaguchi K, Lakshmanan AP, Ma M, Suzuki K, Kodama M, Watanabe K. Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/mas receptor axis. *Int J Biol Sci* 2011;**7**:1077–1092.
122. Sukumaran V, Veeraveedu PT, Lakshmanan AP, Gurusamy N, Yamaguchi K, Ma M, Suzuki K, Kodama M, Watanabe K. Olmesartan medoxomil treatment potently improves cardiac myosin-induced dilated cardiomyopathy via the modulation of ACE-2 and ANG 1-7 mas receptor. *Free Radic Res* 2012;**46**:850–860.
123. Lezama-Martinez D, Flores-Monroy J, Fonseca-Coronado S, Hernandez-Campos ME, Valencia-Hernandez I, Martinez-Aguilar L. Combined antihypertensive therapies that increase expression of cardioprotective biomarkers associated with the renin–angiotensin and kallikrein–kinin systems. *J Cardiovasc Pharmacol* 2018;**72**: 291–295.
124. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME, Johnston CI. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J* 2005;**26**:369–375.

125. Hamming I, van Goor H, Turner AJ, Rushworth CA, Michaud AA, Corvol P, Navis G. Differential regulation of renal angiotensin-converting enzyme (ACE) and ACE2 during ACE inhibition and dietary sodium restriction in healthy rats. *Exp Physiol* 2008;**93**:631–638.
126. Keidar S, Gamliel-Lazarovich A, Kaplan M, Pavlotzky E, Hamoud S, Hayek T, Karry R, Abassi Z. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. *Circ Res* 2005; **97**:946–953.
127. Stoll D, Yokota R, Sanches Aragão D, Casarini DE. Both aldosterone and spironolactone can modulate the intracellular ACE/ANG II/AT1 and ACE2/ANG (1-7)/MAS receptor axes in human mesangial cells. *Physiol Rep* 2019;**7**: e14105.