



# COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection

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## Abstract

COVID-19 pandemic is caused by the novel coronavirus SARS-CoV-2. Angiotensin-converting enzyme 2 (ACE2) is not only an enzyme but also a functional receptor on cell surfaces through which SARS-CoV-2 enters the host cells and is highly expressed in the heart, kidneys, and lungs and shed into the plasma. ACE2 is a key regulator of the renin–angiotensin–aldosterone system (RAAS). SARS-CoV-2 causes ACE/ACE2 balance disruption and RAAS activation, which leads ultimately to COVID-19 progression, especially in patients with comorbidities, such as hypertension, diabetes mellitus, and cardiovascular disease. Therefore, ACE2 expression may have paradoxical effects, aiding SARS-CoV-2 pathogenicity, yet conversely limiting viral infection. This article reviews the existing literature and knowledge of ACE2 in COVID-19 setting and focuses on its pathophysiological involvement in disease progression, clinical outcomes, and therapeutic potential.

**Keywords** Angiotensin-converting enzyme · COVID-19 · SARS-CoV-2 · Tissue damage

## SARS-CoV-2 and ACE2

The acute respiratory tract infection outbreak originated in Wuhan, China, in late 2019, and that spread throughout the globe is caused by a novel coronavirus. Past outbreaks were also caused by highly pathogenic coronaviruses, including the acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. The current outbreak, denominated coronavirus disease 2019 (COVID-19), is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

SARS-CoV-2 is an enveloped virus containing one positive-strand RNA genome that comprises 29.9 kb. SARS-CoV-2 shares 80% identity with SARS-CoV [1], and both viruses use the angiotensin-converting enzyme 2 (ACE2) as a cellular entry receptor [2, 3]. A coronavirus

has four structural proteins: envelope (E), membrane (M), nucleocapsid (N), and spike (S) proteins. The spike protein forms large protrusions from the virus surface, giving the appearance of a crown and, therefore, the name of coronavirus.

The S protein consists of subunits S1 and S2, responsible for the attachment and membrane fusion, respectively. The spike binds to human ACE2 (hACE2) in the cell membrane through the S1 subunit of the receptor-binding domain (RBD). Wrapp et al. [4] documented that SARS-CoV-2 RBD has ~ 10- to 20-fold higher affinity to hACE2 when compared to SARS-CoV RBD binding capacity. Moreover, SARS-CoV-2 RBD binds to soluble hACE2 more strongly than SARS-CoV [3]. This enhanced affinity for hACE2 may contribute to SARS-CoV-2's higher infectivity, as COVID-19 is widespread worldwide and the number of cases is constantly increasing.

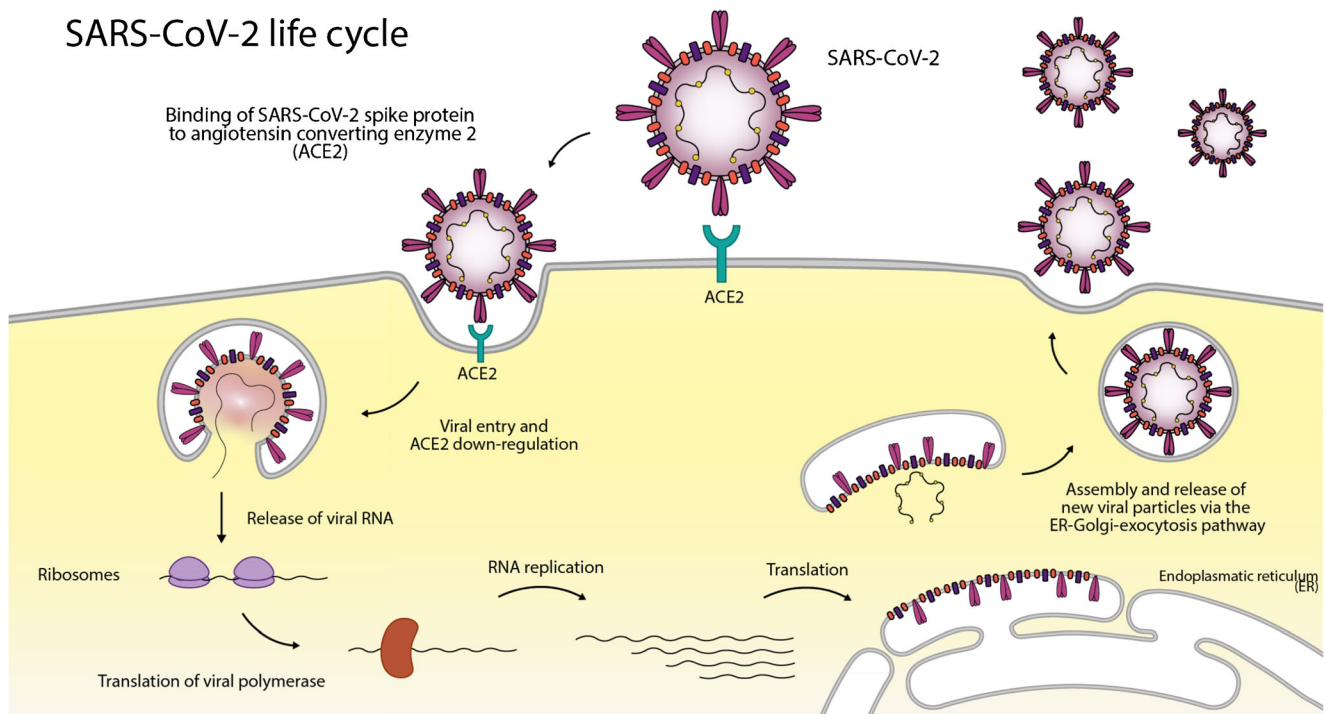
The transmembrane protease serine protease-2 (TMPRSS-2) and ADAM metallopeptidase domain 17 (ADAM17) [5] of the host cell are required for priming the S protein to allow fusion of the viral and host membranes through the S2 subunit [6, 7]. Therefore, SARS-CoV-2 is internalized by endocytosis, and the viral RNA is released for replication and translation by the host cell machinery and further assembly and exocytosis of new viral particles (Fig. 1).

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## SARS-CoV-2 life cycle



**Fig. 1** SARS-CoV-2 life cycle: from binding to ACE2 receptor to shedding

## Angiotensin-converting enzyme 2 and renin–angiotensin–aldosterone system

The renin–angiotensin–aldosterone system (RAAS) is a signalling pathway that acts as a homeostatic regulator of vascular function, as reviewed elsewhere [8]. It has a dynamic control over systemic and local blood flow, blood pressure, natriuresis, and trophic responses to a wide range of stimuli. Therefore, macula densa releases renin within the kidneys upon low intra-tubular sodium concentration and sympathetic nervous stimulation, and then renin converts angiotensinogen into angiotensin I in the liver. Next, angiotensin-converting enzyme (ACE) converts angiotensin I (Ang I) into Ang II, predominantly in the lungs. Then, Ang II stimulates the release of aldosterone from adrenal cortex [9, 10]. Figure 2 shows an overview of the RAAS signalling pathway.

The main axis of the RAAS is composed of ACE/angiotensin II (Ang II)/Ang II receptors AT1 and AT2. Ang II can promote different effects in accordance with the type of receptor, e.g. AT1 receptor binding stimulates the classical effects of Ang II, which include increased oxidative stress, inflammation, fibrosis and vasoconstriction, whereas AT2 receptor binding promotes opposite effects. Moreover, chronic activation of RAAS is associated with Ang II/AT1 deleterious effects, exacerbating the inflammation, fibrosis, apoptosis, antidiuretic hormone (ADH) and aldosterone release, and ultimately water and sodium retention [9, 10]

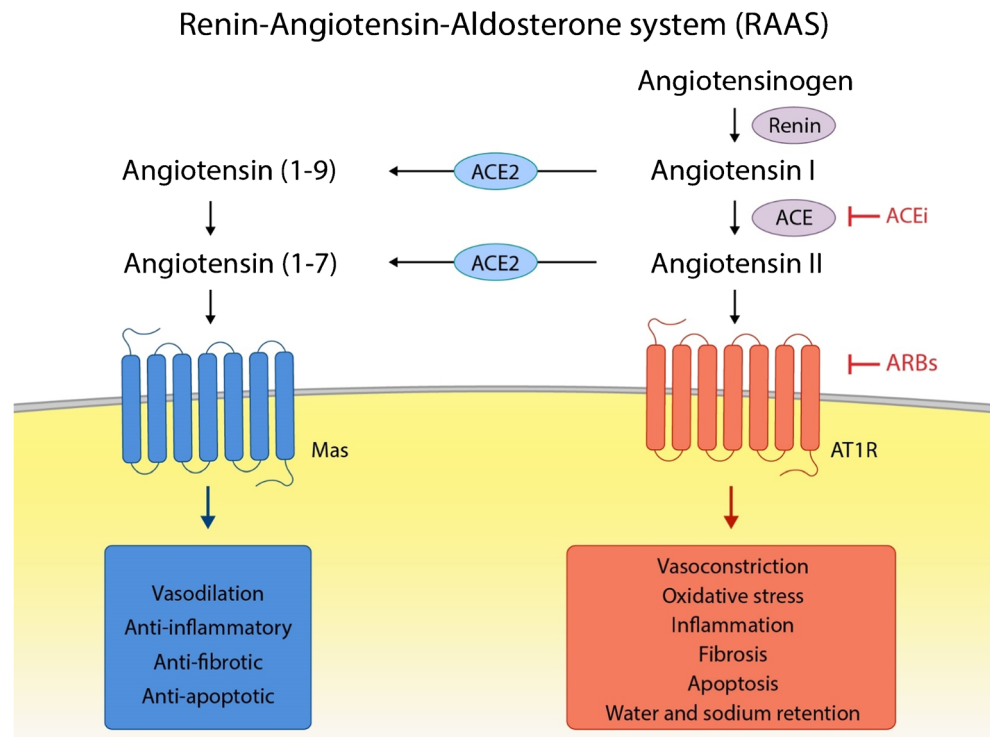
Discovered in 2000 [11] as an ACE homolog with distinguished function, ACE2 is an enzyme that catalyses the cleavage of Ang II into Ang (1-7), Ang I to Ang (1-9), and participates in the hydrolysis of other peptides.

ACE2 has a fundamental role in local and systemic haemodynamics, as its main effect is to lower blood pressure. Ang (1-7), the enzyme main product, binds to the Mas receptor and promotes vasodilation, antioxidant, and antiproliferative effects, thus attenuating Ang II action [12]. Therefore, the ACE2/Ang (1-7)/Mas axis counterbalances the ACE/Ang II/AT1R axis and is an essential regulatory pathway of the RAAS.

Furthermore, ACE2 regulates the ACE action by reducing the amount of Ang II and increasing Ang (1-7). Hence, ACE/ACE2 is essential for the maintenance of tissue liquid volume and electrolyte balance. Pharmacologic therapy with RAAS blockers is widely used for pathologies associated with chronic AT1R activation, especially in systemic hypertension. ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs) promote the accumulation of Ang I and Ang II, respectively, and thus favour the conversion by ACE2 to Ang (1-9) and Ang (1-7) [13].

Importantly, the spike protein is a key determinant of the virus tissue tropism and host range. SARS-CoV-2 competes with Ang II for ACE2 in terms of internalization. The binding, however, blocks ACE2 activity and thus reduces the enzyme expression in the membrane [14]. This may promote RAAS imbalance. Therefore, ACE2 downregulation promotes ACE/ACE2 imbalance and increases ACE/Ang II/AT1R axis

**Fig. 2** Renin–angiotensin–aldosterone system (RAAS): ACE/ACE2 balance, pathophysiological mechanisms, and the impact of the RAAS blockers (ACEi and ARBs). ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers



pathological activation, leading to an increase in Ang II-mediated vasoconstriction and a decrease in Ang (1–7)-mediated vasodilation [10].

Additionally, the kallikrein–kinin system (KKS) is viewed as a natural counterbalance to the RAAS, acting to reduce systemic blood pressure and generation of reactive oxygen species, thus playing a protective role against organ damage in the heart and kidney [15, 16]. The balance between RAAS and KKS affects blood pressure, salt sensitivity, circulating volume, vascular tone, and natriuresis by reducing tubular sodium reabsorption via regulation of the activity of the epithelial sodium channel (ENaC) by bradykinin. Importantly, downregulation of ACE2 following SARS-CoV-2 infection results in an increased bradykinin that can activate the bradykinin (B) 2 receptor to promote vasoconstriction [17]. Dysfunctional RAAS and KKS may ultimately aggravate progression of COVID-19, in particular during cytokine release storm [17].

### ACE2 distribution and COVID-19 manifestations

COVID-19 most common symptoms are fever, dry cough, myalgia, fatigue, and dyspnea. Moreover, other clinical manifestations reported so far are sputum production, headache, abdominal pain, diarrhoea, nausea, vomiting, dizziness, anosmia, dysgeusia, and liver function abnormality [18–21], which

may be explained by the fact that SARS-CoV-2 target several ACE2-expressing tissues.

ACE2 expression is detected in type II alveolar cells (AT2) [22–26], bronchial transient epithelial secretory cells respiratory epithelial cells [27], myocardial cells [22], endothelial cells and artery smooth muscle cells [22], oesophagus epithelial cells [22–24], neurons and glia [28], tongue epithelial cells [29], stomach [23, 26], cholangiocytes [23, 30], adipose tissue [31], pancreatic exocrine glands and islets [32], renal proximal tubule cells [22–25, 33, 34], podocytes [33–35], bladder urothelial cells [22, 26], testis (Leydig and Sertoli cells and spermatogonia) [26, 36, 37], uterus epithelial cells [26], ovary and breast [29], maternal–foetal interface [38], enterocytes from ileum [22–24] and colon [23, 24, 26], and rectum cells [23]. These findings, with the exception of the report by Su et al. [33] that detected ACE2 expression through immunohistochemistry, all used single-cell RNA-seq and protein databases, such as UniProt and Protein Atlas, to detect the receptor expression among different tissues.

Zou et al. [22] linked the ACE2 expression in different organs to their potential risk to SARS-CoV-2 infection. High-risk tissues were defined as containing cells types with > 1% proportion of ACE2 expression and were included in this category: lower respiratory tract (2%), lung (> 1%), heart (> 7.5%), ileum (30%), oesophagus (> 1%), kidney (4%), and bladder (2.4%). To note, stomach and liver samples had < 1% proportion of ACE2-positive cells, indicating that these tissues were considered as low risk for SARS-CoV-2 infection. Of importance, ACE2 expression in nasal epithelium occurs

in an age-dependent manner and may explain, at least in part, the reason why the younger ones have lower incidence of COVID-19, since they exhibited the lowest nasal gene expression of ACE2 [39]. Additionally, the lower expression of ACE2, not only in nasal epithelium but also in bronchial epithelial cells in children, when compared to adults [40], may also explain the non-respiratory COVID-19 symptoms/clinical manifestations in the youngest.

In line with the findings with ACE2, TMPRSS2 expression may also explain the target cell types and clinical manifestation [41]. TMPRSS2 expression is similar to ACE2 expression in many tissues, such as the kidney, liver, testicles, among the gastrointestinal tract, especially in the small intestine, as well as in the lungs and type II alveolar cells [27, 34, 40, 42].

The oral cavity is also at potential risk for SARS-CoV-2 infection [29], as the oral mucosa also expresses ACE2, especially in tongue epithelial cells. This finding is consistent with data that points out the SARS-CoV-2 effects on gustatory and olfactory dysfunctions in COVID-19 patients [43, 44]. Anosmia seen in these cases not only may be attributed to ACE2 expression in the olfactory cavity [45] but also raises questions about SARS-CoV-2 tropism to neural tissues.

To note, ACE2 expression has not been detected in sensory nor olfactory bulb neurons, since neurological manifestation may also be explained by fever [43] or by mucosal congestion, which leads ultimately to nasal obstruction and conductive olfactory loss. However, magnetic resonance (MRI) did not detect abnormal findings in the olfactory bulb and tract and no sign of nasal obstruction was observed [46]. Therefore, anosmia and ageusia may suggest damage to olfactory and gustatory receptors in COVID-19.

ACE2 is expressed in some areas in the brain [28] and neurologic manifestations, such as headache and dizziness, and encephalopathy features detected through MRI [47] support viral capacity to reach the central nervous system (CNS). Based on the SARS-CoV outbreak, Baig et al. [48] proposed a possible pathophysiological mechanism for the involvement of the CNS, where, via haematological dissemination or via the cribriform plate, the virus would be able to break through the blood-brain barrier and to bind to the epithelial ACE2 receptor. Moreover, detection of SARS-CoV-2 in post-mortem samples of frontal lobe [49] and cerebrospinal fluid [50] confirmed the virus neurotropic potential.

Furthermore, evidence of ACE2 expression on oral cavity points to SARS-CoV-2 routes of invasion. Respiratory transmission has been considered the main route for the virus infection; however, new findings may increase our understanding about alternative transmission pathways. For example, ACE2 expression occurs in some sites of the digestive tract and, in fact, SARS-CoV-2's RNA was detected in faecal samples from COVID-19 patients [51]. In addition, detection of the viral RNA in rectal samples for days after nasopharyngeal

swabs tested negative indicates viral shedding for days after IgG seroconversion [52, 53]. Therefore, ACE2 expression and distribution suggest that SARS-CoV-2 may have faecal-oral transmission, yet virus viability in faeces samples requires further investigation.

Regarding respiratory manifestations of COVID-19, most cases present acute lung injury and acute respiratory distress syndrome (ARDS) [54]. The lungs have a high RAAS activity and ACE2 counteraction is essential to homeostasis. AT2 cells synthesize the pulmonary surfactant, which is essential for maintenance of low surface tension at air and liquid interface in alveoli, preventing lung collapse. Alveolar cells (AT1 and AT2) account for 31% of cells in the lungs [23]; however, ACE2 expression is concentrated in a population of AT2 cells that also express viral process-related genes [25]. As SARS-CoV-2 targets AT2 cells, disruption of ACE/ACE2 physiological balance occurs by the infection and local RAAS overactivation leads to increased vascular permeability and oedema. Analyses of lungs obtained during autopsy from patients who died from COVID-19 demonstrated diffuse alveolar damage with necrosis of alveolar lining cells, AT2 hyperplasia, linear inter-alveolar fibrin deposition, marked interstitial oedema with early inter-alveolar organization, and lymphocytic inflammation [55]. In the lungs from patients with COVID-19 and lungs from patients with influenza, the relative counts of ACE2-positive cells for alveolar epithelial cells, endothelial cells, and lymphocyte cells were higher when compared to uninfected individuals. Interestingly, in the lungs from patients with COVID-19 and patients with influenza, similar mean numbers of CD3-positive T cells were found, but CD4-positive T cells were more numerous in the lungs from patients with COVID-19 than in the lungs from patients with influenza, whereas CD8-positive T cells were less numerous. Likewise, neutrophils (CD15<sup>+</sup>) were significantly less numerous adjacent to the alveolar epithelial lining in the COVID-19 group than in the influenza group [55]. In idiopathic pulmonary fibrosis and non-specific interstitial pneumonia, ACE2 was also upregulated within the lungs, as well as in pulmonary arterial hypertension [56]. To note, ACE2 not only functions as a receptor for SARS-CoV-2 entry into cells but also drives the upregulation of genes involved in lung fibrosis [5]. These findings shed the light of our knowledge in pulmonary pathophysiology, susceptibility to SARS-CoV-2 infection, and COVID-19 progression.

Noteworthy is that the reports indicate slightly increased infection rate and significant higher fatality rate in male patients (> 60% vs < 40% women) [19, 57]. ACE2 expression may explain that finding and therefore influence the COVID-19 susceptibility between different ethnicities, genders, and age [31, 56]. ACE2 expression is 3-fold higher in male in comparison to female lung samples, as shown by Zhao et al. through single-cell RNA-seq [25]. Likewise, *in vitro* studies demonstrated that oestrogen may regulate ACE2 expression

in differentiated airway epithelial cells [58]. On the other hand, no gender difference on ACE2 and TMPRSS2 expression was found in salivary glands [59] and bronchial epithelium [60]. Therefore, other factors and behaviours can modulate the enzyme expression, such as smoking, which is more prevalent in men.

Studies using epidemiological data from COVID-19 patients associated smoking to 1.4 times higher chance of developing the severe forms of the disease and 2.4 times higher need of intensive care [61]. Chronic obstructive pulmonary diseases (COPD) present high ACE2 expression in lungs and bronchial epithelium [40, 62–65]. Smoking habits, known to damage the respiratory system and its function, are the leading cause of COPD, which can be aggravated in COVID-19. Smoking can increase ACE2 expression in the lungs and bronchial epithelium [63, 64], so that nicotine may also upregulate ACE2 in neurons and glia and endothelial cells through the nicotinic acetylcholine receptor activation [28]. Thus, smoking can turn these tissues more susceptible to SARS-CoV-2 infection and is a risk factor for COVID-19 pneumonia. Importantly, in people with asthma, ACE-2 and TMPRSS2 gene expression in bronchial epithelial cells was not different from healthy individuals [60], which may explain the lack of association of asthma and COVID-19 severity.

In addition to differences between sexes, ACE2 is highly expressed in the testicles and this raises awareness about the virus effect on fertility and sexual transmission. A single-centre study [66] reported absence of SARS-CoV-2 in semen and male tissues of confirmed COVID-19 patients during acute and post recovery phases. Notably, SARS-CoV-2 transmission by sexual contact is unlikely. Another concern is whether the infection may cause infertility. Yu et al. [67] observed a significant imbalance in male hormones and related this finding to a possible damage to Leydig cells. Nonetheless, longer follow-up is further required and, therefore, the SARS-CoV-2 effect over male fertility remains elusive.

Female reproductive system also expresses ACE2, indicating that it can be a target to SARS-CoV-2. Thus, high-risk group to COVID-19 are pregnant women. Pregnancy increases renal, uterine, and placental ACE2 expression [68, 69] and enhances RAAS activation [70], as observed in murine models. ACE2 exhibits a time-dependent expression in the placenta, so that in late stages of pregnancy, ACE2 is also detected in several foetal tissues [38]. Placental analyses did not show SARS-CoV-2 detection, but disclosed foetal vascular malperfusion in the presence of intramural fibrin deposition and foci of villous stromal–vascular karyorrhexis, and, rarely, infarction [71]. On top of that, 15.4% of pregnant women admitted for delivery tested positive for SARS-CoV-2, yet asymptomatic, which has important clinical implications [72].

Chen et al. [73] did not detect the virus in amniotic fluid, cord blood, breastmilk, and neonatal throat swab samples.

Moreover, COVID-19 symptoms in a small cohort of pregnant women did not differ from non-pregnant patients. Additionally, a case–control study of 225 pregnant women not only unveiled that COVID-19-related symptoms were similar to controls but also reported no difference in the cumulative incidence of early pregnancy loss [74]. However, mothers hospitalized due to COVID-19 pathology had a higher risk of ending their pregnancy via caesarean section, whereas newborns had a higher risk of premature delivery [75].

Currently, there is no evidence of vertical transmission in late pregnancy. Many questions remain, such as what are the effects during early stages of pregnancy or whether SARS-CoV-2 might affect foetal development, due to ACE2 activity reduction [69]. Of importance, the COVID-19 pandemic is still ongoing and further investigation is required.

## Comorbidities and ACE/ACE2 balance disruption

Pre-existing comorbidities are related to a higher risk of developing the severe forms of COVID-19 and higher mortality [19, 76, 77]. Approximately 50% of hospitalized COVID-19 patients have pre-existing medical conditions, including hypertension [78], diabetes mellitus (DM) [79, 80], cardiovascular disease [19, 81, 82], cerebrovascular disease [82], obesity [19, 83, 84], chronic kidney disease [19, 85], smoking [86, 87], and chronic pulmonary disease [19, 87]. In addition, increasing age and male sex are at greater risk of mortality during hospitalization [19, 88, 89].

Likewise, uremic patients under chronic haemodialysis exhibited an increase in ACE and Ang II plasmatic levels, whereas ACE2 and Ang (1–7) levels were lower when compared to controls [90]. That imbalanced ACE/ACE2 ratio was more severe in the haemodialysis patients with cardiovascular disease.

These pre-existing comorbidities are associated with chronic endothelial dysfunction, so that SARS-CoV-2 may aggravate those conditions due to endotheliitis, apoptosis, and lymphocytic and mononuclear infiltrating cells [55, 91]. Therefore, SARS-CoV-2-mediated endothelial dysfunction and impaired vascularization, induced by chronic diseases, accelerate vascular disease and prevent recovery from ischemic insults. Pre-existing comorbidities may also be directly associated to higher rates of SARS-CoV-2 organ tropism, as demonstrated in post-mortem analyses of the lungs, pharynx, kidneys, liver, and heart [35]. To further determine the SARS-CoV-2-mediated cytopathic effects on different organs, autopsy studies provided compelling evidences on diffuse alveolar damage with activated type II pneumocytes, fibroblasts, protein-rich exudate, hyaline membrane, and infiltrating lymphocytes in the wall of pulmonary arteries [55, 92, 93]. In

advanced stages of COVID-19, squamous metaplasia and fibrosis occurred, which is consistent with data showing that SARS-CoV-2 infection *in vitro* increases the expression of factors associated with remodelling and fibrosis [5]. Importantly, the most frequent cause of death was pneumonia, followed by pulmonary artery embolisms combined with pneumonia. Diffuse alveolar damage is associated with SARS-CoV-2 detection in pulmonary pneumocytes and ciliated airway cells by immunohistochemistry (polyclonal antibody against nucleocapsid protein), so that in the stage of organizing pneumonia, viral detection was not observed [94].

In the kidneys, post-mortem analyses disclosed proximal acute tubule injury manifested as the loss of brush border, dilatation of the tubular lumen with cellular debris, vacuolar degeneration, and occasionally even frank necrosis and detachment of epithelium with loss of tubular basement membrane [33]. Likewise, viral inclusion bodies were found in peritubular space and in endothelial cells of the glomerular capillary loops [33, 35, 91], as assessed by electron microscopy. In small bowel intestine, dominant mononuclear cell infiltrates within the intima along the lumen vessels associated with apoptosis of endothelial cells were verified in resection specimen [91]. To note, small bowel lesions may be associated to mucosal necrosis, transmural inflammation, and ischemic injury due to superior mesenteric artery thrombosis and viral particles clustered within membrane-bound cisternal spaces in the enterocytes [95].

Whether those histological findings are broadly determined by complement associated microvascular injury and thrombosis [96] or by apoptosis mediated by ORF3a protein of SARS-CoV-2 [97], further investigation are warranted to investigate the crosstalk with RAAS system and the most appropriate clinical approaches for combating the COVID-19.

Next, to gain insights into the interaction between RAAS activation and SARS-CoV-2 pathogenicity, we will discuss the current evidence of RAAS modulation in healthy and diseased individuals.

The RAAS system is a haemodynamic and biological system, which plays a crucial role in the regulation of blood pressure, plasma sodium and potassium, cell proliferation, fibrosis, and oxidative stress. As previously described, the liver synthesizes angiotensinogen and renin, which is produced by juxta-glomerular apparatus, and converts angiotensinogen into Ang I. Next, ACE converts Ang I into Ang II, whereas ACE2 converts Ang I into Ang (1–9) and Ang II into Ang (1–7) [9]. Ang II attaches to its receptor (Ang II type 1 receptor or AT1R or to ATR2), while Ang (1–7) binds to Mas receptor, leading to vasodilation and decreasing inflammation, cell proliferation, hypertrophy, and fibrosis [98]. To note, Mas and ATR2 are key receptors that possess counter-regulatory properties in relation to Ang II/ATR1 binding, attenuating its effect on vasoconstriction, sodium retention, inflammation, and fibrosis (Fig. 2).

Noteworthy, SARS-CoV-2 enters into host cells after binding to ACE2 receptor [99], and both SARS-CoV-2 and ACE2 are internalized by endocytosis, so that surface ACE2 is then downregulated, resulting in unopposed Ang II accumulation [9]. There are two isoforms of ACE2, a circulating or soluble isoform (sACE2) and the other isoform is not only tissue-specific, but also cell-specific. As mentioned previously, ACE2 expression is broad, including the heart, kidneys, and lungs.

When tissues are damaged, ACE2 is released from cytoplasmic membrane to plasma and, therefore, can be used as a surrogate marker of mortality. Thus, plasmatic ACE2 is increased in individuals with coronary artery disease [56, 100], myocardial infarction [56], heart failure [101], atrial fibrillation [56, 102], and aortic stenosis [103–105]

In line with these findings, higher levels of plasma sACE2 were found in men when compared to women and in older individuals, with the age association more pronounced in women [105]. In addition, higher levels of sACE2 were detected in post-menopausal women, compared to pre-menopausal women. In other diseases, such as metabolic syndrome (effect stronger in men) and its components, such as obesity, hypertension, insulin resistance, and dyslipidaemia displayed elevated plasma sACE2 levels when compared to controls [105]. Importantly, the impact of sex on the immune response may provide a broader explanation of the differences in COVID-19-related mortality in men [104]. ACE2 gene is located on X chromosomes, which is important for its ability to recognize the SARS-CoV-2. Therefore, SARS-CoV-2 RBD binds to ACE2 more frequently in men. The same SARS-CoV-2 RBD can be recognized by ACE2 on either of the two X chromosomes in females, but the chance that the same residue sequences of ACE2 on the second chromosome also perfectly binds to the SARS-CoV-2 RBD is low, allowing unbound ACE2 to catalytically cleave Ang II to form Ang (1–7), and thus decrease COVID-19-related clinical complications [104]. Interleukin-1 receptor-associated kinases (IRAKs) and inhibitors of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) kinases (IKKs) are also encoded on the X chromosome and may confer an advantage in responding to and resolving SARS-CoV-2 infections in females through Toll-like receptor 4 (TLR4) signalling pathway. The X chromosomes together with sex hormones act in concert to increase the production of interferon by dendritic cells contributing to the enhanced TLR7-mediated response in females. Oestrogen may also contribute to higher basal immunoglobulin levels, larger numbers of circulating and resident CD4/CD8 T-lymphocyte ratio, reduced levels of fibrinogen and plasminogen-activator inhibitor type 1 (PAI-1), and increased levels of antithrombin III. These findings have important biological implication in COVID-19 progression, in particular the cytokine release storm and thromboembolism [104].

To further substantiate these findings, a recent cross-sectional study, including two larger European and Scottish cohorts of male and female individuals diagnosed with heart failure and taking ACE inhibitors, ARBs, or mineralocorticoid receptor antagonists, demonstrated that in both cohorts, male gender was the only predictor of elevated plasma concentration of ACE2 activity [106]. In the European cohort, those drugs failed to predict plasma ACE2 activity. Conversely, in the Scottish cohort, ACEi and ARB use were independent predictors of lower plasma ACE2, while the use of a mineralocorticoid receptor antagonist was an independent predictor of higher plasma ACE2 concentrations.

Importantly, several lines of evidence support ACE and ACE2 regulation in a manner of tissue and organ dependent. Thus, analyses of ACE and ACE2 expression in the heart of individuals with aortic stenosis and low left ventricular ejection fraction unveil higher expression of both genes in cardiomyocytes when compared to healthy controls [107], as well as in patients with dilated cardiomyopathy and arrhythmia [56]. Nonetheless, diseased heart exhibited a lower expression of ACE2, yet in endothelial cells, ACE2 expression was similar to healthy individuals [107]. After ARB administration, ACE2 did not change, as opposed to the increase in ACE2 and ACE/ACE2 ratio in cardiomyocytes after ACEi treatment [107].

In individuals with type 2 DM, ACE2 expression is elevated in the pancreas, liver, and adipose tissue, whereas in diseased liver (cirrhosis, dysplasia, steatosis, and steatohepatitis), ACE2 is equally increased [56]. Similar findings were reported in pneumonia-related diseases, such as interstitial pneumonia, tuberculosis, idiopathic pulmonary fibrosis, and pulmonary arterial hypertension [56]. Collectively, these data indicate a higher risk of severe COVID-19 in patients with those comorbidities.

In line with the findings of cell type-specific expression in the other tissues, ACE and ACE2 protein expression revealed higher levels of ACE in either tubular or glomerular compartments, which was associated with a decrease in ACE2 expression in human kidney samples from individuals with type 2 diabetic kidney disease and hypertension [108]. Additionally, in chronic kidney disease, both ACE and ACE2 expression decreased.

Within the kidneys of patients with COVID-19, the cytopathic effects of SARS-CoV-2 in podocytes and proximal straight tubule cells may cause acute kidney injury, since these compartments express high levels of ACE2 and TMPRSS2 [34]. Unexpectedly, podocytes and proximal tubular cells of western individuals displayed higher expression of ACE2 and TMPRSS2, which may explain, at least in part, the most frequent occurrence of kidney injury in western individuals. Likewise, further investigation is required to correlate ACE2 expression in other cells within the damaged kidneys, such as T and B lymphocytes, NK cells and monocyte/macrophage. In

human kidneys with type 2 diabetic kidney disease and hypertension, an imbalance of RAAS was also noticed by an upregulation of ACE and a downregulation of ACE2 in glomeruli and tubular epithelial cells, as reviewed elsewhere [108]. As kidney disease progresses, both ACE and ACE2 expression decreases. The clinical implications of these findings require further elucidation in diabetic and hypertensive individuals with COVID-19. As a result of the viral tropism to the kidneys, acute renal injury was found to be the second most observed COVID-19 complication in the USA (22.2%) [77] and China (25.2%) [67]. Thus, there is compelling evidences that patients with acute kidney injury at admission exhibit activation of RAAS, as documented by higher plasmatic renin and aldosterone levels and lower urinary sodium levels in patients with acute kidney injury [109], as well as the association of plasmatic aldosterone and C-reactive protein levels and COVID-19 severity [110]. Since hypokalaemia was found to be prevalent in patients with COVID-19 pneumonia (~ 30%) and is an indicator of RAAS activity, it was suggested that hypokalaemia could be used as a surrogate marker of COVID-19 progression and an independent risk factor for invasive mechanical ventilation requirement [111]. Conversely, mean serum concentrations of ACE2, Ang II, and aldosterone, as well as potassium levels and blood pressure, were found to be similar in patients with COVID-19 and the control group [112].

To provide further evidence that RAAS blockers were not harmful in COVID-19 setting, several studies were set out to analyse whether the use of ACEi and ARBs and other antihypertensive drugs were associated with COVID-19 progression. These studies documented that those drugs were not a risk factor for the likelihood of positive test for COVID-19 or for severe COVID-19 [113–115], even after adjustment for age and gender. RAAS blockers decreased the risk of hospitalization in 47% for diabetic patients [116], and the risk of severity in 29% and the mortality in 43% for hypertensive patients [117]. Although hypertensive patients may exhibit COVID-19 progression, the lack of antihypertensive treatment in that setting imposes an additional risk [118]. Therefore, RAAS blockers are protective in COVID-19 pandemic, so that they decrease mortality by 35% [118]. An important meta-analysis comprising 28,872 participants on ACEi/ARBs with COVID-19 documented a trend to lower risk of death or critical events when RAAS inhibition was used for any conditions [119]. In particular, hypertensive individuals had significantly 34% reduction in death and 33% reduction in the combination of death/critical outcomes. Therefore, it is strongly encouraged that patients continue with RAAS blockers during the COVID-19 pandemic.

Another clinical condition that play a crucial role in COVID-19 pandemic is obesity [19, 76, 77, 120]. Thus, obesity is associated with COVID-19 progression (odds ratio for grade I obesity ranged from 1.30 to 1.80 [83, 121] and for

grade II obesity from 3.60 to 7.36 [83, 122]). As expected, obesity in young Chinese patients (< 45 years old) was associated with an increased COVID-19 mortality [123]. In American patients with body mass index (BMI) greater than 40 kg/m<sup>2</sup>, the adjusted odds ratio for death was 5.10 for those with age less than 50 years old and 1.60 for those with more than 50 years old [84]. In obese individuals with COVID-19, inflammatory markers were higher when compared to non-obese individuals, as well as the oxygenation parameters at admission and the requirement of higher volumes of oxygen and a long period to achieve oxygen weaning [124] and the higher rates of invasive mechanical ventilation [122]. Differences in ACE2 bronchial epithelial expression between patients with chronic pulmonary disease who are overweight (mean BMI 29 kg/m<sup>2</sup>) when compared to those not overweight (mean BMI 21 kg/m<sup>2</sup>) may account for the higher rates of COVID-19 in obese individuals [65]. Therefore, obese patients present an increased risk of critical illness leading to intensive care unit admission and death (risk relative of 1.58, even after adjusting for age and race) [125].

On the other hand, SARS-CoV-2 may infect adipose tissue and contribute to immune activation and ultimately to cytokine release storm [126]. Therefore, obesity is a risk factor (6-fold) for greater severity of COVID-19 patients with metabolic associated fatty liver disease, even after adjustment for age, gender, DM, hypertension, smoking, and dyslipidaemia [127]. That effect can be more evident in patients under 60 years old, which can explain the adverse clinical outcomes in the youngest. In addition, BMI is associated with epicardial adipose tissue volume. Therefore, ACE2 and inflammatory cytokines (TNF- $\alpha$  and IL-6) in association with macrophage polarization were detected at higher levels in epicardial adipose tissue in obese patients with heart failure and preserved ejection fraction [128]. Likewise, knockout mice for ACE2 exhibited an exacerbated epicardial adipose tissue inflammation and lipotoxicity, which was reversed by Ang (1–7) administration [128]. We may speculate, therefore, that in the COVID-19 setting, the downregulation of ACE2 induced by the SARS-CoV-2 may contribute to epicardial adipose tissue inflammation and ultimately to cardiac complications. It is equally anticipated that, when SARS-CoV-2 infection increases the levels of Ang II and decreases the levels of Ang (1–7), a subsequent downregulation in the activity of pyruvate dehydrogenase complex occurs [129]. That reduction may lead ultimately to a decrease in the rate of glycolysis, resulting in uncoupling between glycolysis and glucose oxidation. This uncoupling induces intracellular acidosis and energy depletion. Importantly, diabetic individuals who are overweight express high levels of ACE2 in adipose tissue when compared to those who are also diabetic but present weight stability [56]. Taken together, all these findings highlight mechanistic explanation for higher rates of COVID-19 progression in DM setting.

Additionally, the expression of ACE2 is upregulated in adipocytes of patients with obesity and DM, indicating that adipose tissue is a potential target and SARS-CoV-2 reservoir [130]. Whether the pulmonary adipocytes possess the capacity of transdifferentiating into myofibroblasts and whether these pulmonary lipofibroblasts located in the alveolar interstitium identically contribute to lung fibrosis remain elusive [130]. However, further evidence is required to verify the therapeutic potential of thiazolidinedione in COVID-19. These drugs may stabilize lipofibroblasts in their inactive state and may decrease fibrosis, yet the crosstalk with ACE2 and inflammation inhibition in adipose tissue has not been established.

Milne et al. [120] verified the effect of RAAS blockers on ACE2 gene expression in lung tissue samples and found no association between ARB use and ACE2 expression, whereas ACEi use was associated with both lower TMPRSS2 and ACE2 expression. That finding may be attributed to the fact that ACEi reduce Ang II conversion and consequently decrease ACE2 substrate availability.

### Monoclonal antibody-based therapy targeting the spike protein

Future directions encompass ACE2 as a therapeutic target in COVID-19 setting, which may slow the virus entering spreading. These strategies comprise the delivery of soluble form of ACE2 (sACE2) [131] and the blocking of the interaction between the SARS-CoV-2 and hACE2 [132].

Of importance, ACE2 exists in two isoforms in the body, e.g. the one that is anchored in the plasma membrane of cells and the soluble (sACE2). As sACE2 contains the RBD to the virus binding, but is not attached to the membrane, delivering sACE2 will abrogate SARS-CoV-2 entering the cells. As documented, administration of soluble human recombinant ACE2 (rACE2; half-life of 8.5 h) into mice resulted in the increase of systemic ACE2 activity, but not in tissues such as heart and kidney [133]. In addition, soluble human rACE2 prevented the hypertensive effect of Ang II, and this was associated with both decrease in Ang II by enhancing its degradation and an increase in Ang (1–7) levels in the plasma. These findings indicate that targeting ACE2 activity in the plasma may have important biological implications in states of Ang II overactivity. Therefore, pursuing sACE2 activity may represent a useful strategy to reduce SARS-CoV-2 infectivity.

Likewise, investigation of antibodies with potent anti-SARS-CoV-2 neutralization activity that correlates with their competitive capacity with ACE2 for the receptor-binding domain (RBD) of the viral spike protein binding has important clinical implications.

Crystal structure analysis of the most potent antibodies (P2C-1F11, P2B-2F6, and P2C-1A3) verified competition with ACE2 binding, indicating that blocking the RBD and



ACE2 is a surrogate for virus neutralization [134]. Likewise, CV30 monoclonal antibody (mAb) binds to an epitope that overlaps with the hACE2 RBD providing a structural basis for its neutralization [135]. More importantly, competition with ACE2, rather than binding affinity, may better predict antibody potency. Two other monoclonal antibodies (B38 and H4) may act synergistically to block the RBD of the viral spike protein and the cellular response receptor ACE2, not only *in vitro*, but also in transgenic mice expressing hACE2 [136]. In these animals, both antibodies decreased virus replication and ameliorated lung injury. Other monoclonal antibodies, obtained from convalescent COVID-19 individuals, and with the capacity to recognize distinct epitopes of the RBD of the viral spike protein (CA1 e CB6), were equally potent to neutralize the SARS-CoV-2 *in vitro* [137]. Similarly, CB6 antibody inhibited SARS-CoV-2 infection in rhesus monkeys at both prophylactic and treatment settings.

Likewise, after screening a large panel of human mAbs that target the spike protein, two antibodies, COV2-2196 and COV2-2130, recognized the non-overlapping epitopes on RBD and bound simultaneously to the S protein inducing virus neutralization in a synergistic manner [138]. In both mice and rhesus macaques, these antibodies prevented these animals from SARS-CoV-2 infection.

The epitope specificity may also play a role in antibody functional activity. Therefore, mAb directed to RBD-A epitope (CC6.29 and CC6.30) showed the highest neutralization potency when compared to mAb directed to non-RBD epitopes on the spike protein [139]. Importantly, the antibodies that did not bind to RBD and were directed to non-RBD epitopes on the S protein all showed poor neutralization potencies.

A significant concern for any antibody, drug, or vaccine is the potential for SARS-CoV-2 acquiring resistance due to rapid mutation, a common finding in RNA virus. Therefore, a cocktail therapy may provide a powerful way to minimize mutational escape by SARS-CoV-2. Baum et al. documented the therapeutic potential of antibody cocktails using two antibodies (REGN10987 + REGN 10933), which possessed the highest capacity to bind to distinct and non-overlapping regions of the viral target, e.g. the RBD of the spike protein [140].

To note, numerous mAbs and mAb cocktails provided evidence of prophylaxis [136, 138–141]. Thus, 1B07 mAb, a chimeric mouse Fv-human Fc (IgG1) antibody, when used pre-SARS-CoV-2 exposure in adenovirus-hACE2 mice, reduced viral RNA load in the lungs and in extrapulmonary sites, including the heart and spleen, and ameliorated lung pathology and inflammatory markers, such as IL-6, Cxcl10, Ccl2, Ccl5, and Cxcl11 [141]. Moreover, both prophylactic and therapeutic potentials of mAbs are desirable. For example, IgG1 ab1, an antibody with high affinity and specificity to SARS-CoV-2 RBD and highly competitive with ACE2,

prevented mice from disease burden by decreasing viral shedding in lung tissue when used prophylactically or therapeutically [142]. The mechanism of neutralization could also involve antibody-dependent cellular toxicity.

As an alternative, a strategy to enhance mAb potential is the biparatopic therapy, the 89C8-ACE2 [143], which combines a monoclonal antibody (89C8) that recognizes the N-terminal domain (NTD) of the S protein, and the ectodomain of ACE2, which binds to the RBD. This approach showed potent neutralizing activity in pseudovirus and live virus assays. In the last one, the construct had higher neutralizing capacity when compared to the ACE2-Fc and the mAb alone, which showed weak neutralizing ability.

Overall, these results suggest antibodies with potent anti-SARS-CoV-2 neutralization activity deserve further clinical translation.

Recently, an interim analysis of a phase 2 trial demonstrated the therapeutic potential of the LY-CoV55 neutralizing antibody in patients with mild and moderate COVID-19 [144]. The main findings were faster viral clearance in patients who received the dose of 2800 mg, lower incidence of hospitalization, mainly in those who were 65 years of age or older and among those with a body mass index of 35 kg/m<sup>2</sup> or more, and lower symptom burden. Importantly, the LY-CoV55 antibody exhibited a safety profile.

## ACE1/ACE2 gene polymorphisms

Whether genetic variability of ACE1/ACE2 explains the clinical course of COVID-19 among various ethnic populations, the studies remain elusive.

Therefore, four missense mutations showed significant minor allele frequency of ACE2 between Asians and Caucasians [145]. Two of these variants (K26R and I468W) may affect binding between S protein of the SARS-CoV-2 and hACE2 receptor. ACE2 expression between Asians and other seven populations disclosed a difference in male (two populations) and female (three populations) individuals.

When evaluating ACE2 genetic variation in COVID-19 progression, the variant rs2285666 of ACE2 was not associated with the disease outcome [89].

Nevertheless, several studies have demonstrated strong association between ACE1-insertion/deletion (I/D) and COVID-19. ACE1-DD carriers have higher blood levels of ACE, approximately twice when compared to ACE1-II individuals, and have been associated with hypertension, ARDS, and in-hospital mortality [146–148]. Therefore, the deletion allele is associated with COVID-19 progression [89] and SARS-CoV-2 infection rate and mortality [147], while the ACE1-II genotype negatively correlates with infection rate and mortality [149]. However, a meta-

analysis (48,758 healthy subjects from 30 different countries) significantly associated ACE1-I/D allele frequency ratio with the increase in the recovery rate, but not with mortality [150].

Moreover, ACE1-I/D polymorphisms could explain the clinical course of COVID-19 among various ethnic populations. The distribution of the D/D, I/D, and I/I genotypes frequency ratios in African Americans (29%, 60%, and 11%, respectively), Indians (19%, 50%, and 31%, respectively), and whites (29%, 40%, and 31%, respectively) were significantly different. The frequency of the deletion allele among African Americans, Indians, and whites (0.59, 0.49, and 0.44, respectively) was also significantly different [151]. These findings could explain the differences in cardiovascular disease, in particular in African Americans, who exhibit the higher frequency of the deletion allele. Whether these evidences could explain COVID-19 progression in different populations, further information is warranted.

Overall, the significance of the findings of ACE and ACE2 allele frequency ratio require further in vitro and functional studies to clarify their role in COVID-19 studies.

## Conclusions

ACE2 expression and its modulation by conditions and underlying comorbidities may increase tissue susceptibility to COVID-19. Moreover, SARS-CoV-2 disrupts the ACE/ACE2 physiological balance and activates the Ang II/AT1R pathways, leading to severe complications of the disease. Thus, strategies that target the overactivation of RAAS and its deleterious effects in COVID-19 setting deserve further investigation.

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## Compliance with ethical standards

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## References

- 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 (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579:270–273
- Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426:450–454
- Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, Zhou Y, Du L (2020) Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cellular & Molecular Immunology* 17:613–620
- Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, Graham BS, McLellan JS (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367:1260
- Xu J, Xu X, Jiang L, Dua K, Hansbro PM, Liu G (2020) SARS-CoV-2 induces transcriptional signatures in human lung epithelial cells that promote lung fibrosis. *Respiratory Research* 21:182
- Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A, Müller MA, Drosten C, Pöhlmann S (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181:271–280.e8
- Li F (2016) Structure, function, and evolution of coronavirus spike proteins. *Annual Review of Virology* 3:237–261
- Iwai M, Horiuchi M (2009) Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-Mas receptor axis. *Hypertension Research: Official Journal of the Japanese Society of Hypertension* 32:533–536
- Silhol F, Sarlon G, Deharo JC, Vaïsse B (2020) Downregulation of ACE2 induces overstimulation of the renin-angiotensin system in COVID-19: should we block the renin-angiotensin system? *Hypertension Research* 43:854–856
- Murray E, Tomaszewski M, Guzik TJ (2020) Binding of SARS-CoV-2 and angiotensin-converting enzyme 2: clinical implications. *Cardiovascular Research* 116:e87–e89
- Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ (2000) A human homolog of angiotensin-converting enzyme. *Journal of Biological Chemistry* 275:33238–33243
- Tikellis C, Bernardi S, Burns WC (2011) Angiotensin-converting enzyme 2 is a key modulator of the renin-angiotensin system in cardiovascular and renal disease. *Current Opinion in Nephrology and Hypertension* 20:62–68
- Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD (2020) Renin-angiotensin-aldosterone system inhibitors in patients with COVID-19. *New England Journal of Medicine* 382:1653–1659
- Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, Simmons G, Hofmann H, Kuri T, Weber F, Eichler J, Drosten C, Pöhlmann S (2010) Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. *Journal of Virology* 84:1198
- Madeddu P, Emanuelli C, El-Dahr S (2007) Mechanisms of disease: the tissue kallikrein-kinin system in hypertension and vascular remodeling. *Nat Clin Pract Nephrol* 3(4):208–221

16. Rhaleb N-E, Yang X-P, Carretero OA (2011) The kallikrein-kinin system as a regulator of cardiovascular and renal function. *Compr Physiol* 1(2):971–993
17. Karmouty-Quintana H, Thandavarayan RA, Keller SP, Sahay S, Pandit LM, Akkanti B (2020) Emerging mechanisms of pulmonary vasoconstriction in SARS-CoV-2-induced acute respiratory distress syndrome (ARDS) and potential therapeutic targets. *Int J Mol Sci* 21(21):E8081
18. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 323:1061–1069
19. Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, Holden KA, Read JM, Dondelinger F, Carson G, Merson L, Lee J, Plotkin D, Sigfrid L, Halpin S, Jackson C, Gamble C, Horby PW, Nguyen-Van-Tam JS, Ho A, Russell CD, Dunning J, Openshaw PJ, Baillie JK, Semple MG (2020) Features of 20133 UK patients in hospital with COVID-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. *BMJ* 369:m1985
20. Lin L, Jiang X, Zhang Z, Huang S, Zhang Z, Fang Z, Gu Z, Gao L, Shi H, Mai L, Liu Y, Lin X, Lai R, Yan Z, Li X, Shan H (2020) Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 69:997
21. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu B (2020) Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurology* 77:683–690
22. Zou X, Chen K, Zou J, Han P, Hao J, Han Z (2020) Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Frontiers of Medicine* 14:185–192
23. Qi F, Qian S, Zhang S, Zhang Z (2020) Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochemical and Biophysical Research Communications* 526:135–140
24. Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, Li Z, Cui X, Xiao J, Zhan J, Meng T, Zhou W, Liu J, Xu H (2020) Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut* 69:1010–1018
25. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W (2020) Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. *American Journal of Respiratory and Critical Care Medicine* 202:756–759
26. Zhang J, Wu Y, Wang R, Lu K, Tu M, Guo H, Xie W, Qin Z, Li S, Zhu P, Wang X (2020) Bioinformatic analysis reveals that the reproductive system is potentially at risk from SARS-CoV-2. Preprints
27. Lukassen S, Chua RL, Trefzer T, Kahn NC, Schneider MA, Muley T, Winter H, Meister M, Veith C, Boots AW, Hennig BP, Kreuter M, Conrad C, Eils R (2020) SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. *The EMBO Journal* 39:e105114–e105114
28. Kabbani N, Olds JL (2020) Does COVID19 infect the brain? If so, smokers might be at a higher risk. *Molecular Pharmacology* 97:351–353
29. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, Li T, Chen Q (2020) High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *International Journal of Oral Science* 12:8
30. Chai X, Hu L, Zhang Y, Han W, Lu Z, Ke A, Zhou J, Shi G, Fang N, Fan J, Cai J, Fan J, Lan F (2020) Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. *bioRxiv*
31. Chen J, Jiang Q, Xia X, Liu K, Yu Z, Tao W, Gong W, Han J-DJ (2020) Individual variation of the SARS-CoV2 receptor ACE2 gene expression and regulation. *Aging Cell* 19:e13168
32. Liu F, Long X, Zou W, Fang M, Wu W, Li W, Zhang B, Zhang W, Chen X, Zhang Z (2020) Highly ace2 expression in pancreas may cause pancreas damage after SARS-CoV-2 infection. *Clin Gastroenterol Hepatol* 18(9):2128–2130.e2
33. Su H, Yang M, Wan C, Yi L-X, Tang F, Zhu H-Y, Yi F, Yang H-C, Fogo AB, Nie X, Zhang C (2020) Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney International* 98:219–227
34. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG (2020) Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Medicine* 46:1114–1116
35. Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, Chilla S, Heinemann A, Wanner N, Liu S, Braun F, Lu S, Pfeifferle S, Schröder AS, Edler C, Gross O, Glatzel M, Wichmann D, Wiech T, Kluge S, Püeschel K, Aepfelbacher M, Huber TB (2020) Multiorgan and renal tropism of SARS-CoV-2. *New England Journal of Medicine* 383:590–592
36. Douglas GC, O'Bryan MK, Hedger MP, Lee DKL, Yarski MA, Smith AI, Lew RA (2004) The novel angiotensin-converting enzyme (ACE) homolog, ACE2, is selectively expressed by adult Leydig cells of the testis. *Endocrinology* 145:4703–4711
37. Wang Z, Xu X (2020) scRNA-seq profiling of human testes reveals the presence of the ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, Leydig and Sertoli cells. *Cells* 9:920
38. Li M, Chen L, Zhang J, Xiong C, Li X (2020) The SARS-CoV-2 receptor ACE2 expression of maternal-fetal interface and fetal organs by single-cell transcriptome study. *PLOS ONE* 15:e0230295
39. Bunyavanich S, Do A, Vicencio A (2020) Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *JAMA* 323:2427–2429
40. Saheb Sharif-Askari N, Saheb Sharif-Askari F, Alabed M, Temsah M-H, al Heialy S, Hamid Q, Halwani R (2020) Airways expression of SARS-CoV-2 receptor, ACE2, and TMPRSS2 is lower in children than adults and increases with smoking and COPD. *Molecular Therapy. Methods & Clinical Development* 18:1–6
41. Vaarala MH, Porvari KS, Kellokumpu S, Kyllönen AP, Vihko PT (2001) Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. *The Journal of Pathology* 193:134–140
42. Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfeifferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, Niemeyer D, Schneider H, Drosten C, Pöhlmann S (2011) Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *Journal of Virology* 85:4122
43. Lechien JR, Chiesa-Estomba CM, de Siaty DR, Horoi M, Ie Bon SD, Rodriguez A, Dequanter D, Blecic S, el Afia F, Distinguin L, Chekkoury-Idrissi Y, Hans S, Delgado IL, Calvo-Henriquez C, Lavigne P, Falanga C, Barillari MR, Cammaroto G, Khalife M, Leich P, Souchay C, Rossi C, Joume F, Hsieh J, Edjlali M, Carlier R, Ris L, Lovato A, de Filippis C, Coppee F, Fakhry N, Ayad T, Saussez S (2020) Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *European Archives of Oto-Rhino-Laryngology* 277:2251–2261
44. Vaira LA, Salzano G, Deiana G, de Riu G (2020) Anosmia and ageusia: common findings in COVID-19 patients. *The Laryngoscope* 130:1787
45. Brann DH, Tsukahara T, Weinreb C, Lipovsek M, van den Berge K, Gong B, Chance R, Macaulay IC, Chou H, Fletcher R, Das D,

- Street K, de Bezieux HR, Choi Y-G, Rizzo D, Dudoit S, Purdom E, Mill JS, Hachem RA, Matsunami H, Logan DW, Goldstein BJ, Grubb MS, Ngai J, Datta SR (2020) Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Science Advances* 6:eabc5801
46. Galougahi MK, Ghorbani J, Bakhshayeshkaram M, Naeini AS, Haseli S (2020) Olfactory bulb magnetic resonance imaging in SARS-CoV-2-induced anosmia: the first report. *Academic Radiology* 27:892–893
  47. Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, Collange O, Boulay C, Fafi-Kremer S, Ohana M, Anheim M, Meziani F (2020) Neurologic features in severe SARS-CoV-2 infection. *New England Journal of Medicine* 382:2268–2270
  48. Baig AM, Khaleeq A, Ali U, Syeda H (2020) Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. *ACS Chemical Neuroscience* 11:995–998
  49. Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednický J, Sordillo EM, Fowkes M (2020) Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Journal of Medical Virology* 92:699–702
  50. Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, Takamino J, Ueno M, Sakata H, Kondo K, Myose N, Nakao A, Takeda M, Haro H, Inoue O, Suzuki-Inoue K, Kubokawa K, Ogihara S, Sasaki T, Kinouchi H, Kojin H, Ito M, Onishi H, Shimizu T, Sasaki Y, Enomoto N, Ishihara H, Furuya S, Yamamoto T, Shimada S (2020) A first case of meningitis/encephalitis associated with SARS-coronavirus-2. *International Journal of Infectious Diseases: IJID : Official Publication of the International Society for Infectious Diseases*. 94:55–58
  51. Amirian ES (2020) Potential fecal transmission of SARS-CoV-2: Current evidence and implications for public health. *International Journal of Infectious Diseases* 95:363–370
  52. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirgmaier K, Drosten C, Wendtner C (2020) Virological assessment of hospitalized patients with COVID-2019. *Nature* 581:465–469
  53. Smyk W, Janik MK, Portincasa P, Milkiewicz P, Lammert F, Krawczyk M (2020) COVID-19: focus on the lungs but do not forget the gastrointestinal tract. *European Journal of Clinical Investigation* e13276
  54. Argenziano MG, Bruce SL, Slater CL, Tiao JR, Baldwin MR, Barr RG, Chang BP, Chau KH, Choi JJ, Gavin N, Goyal P, Mills AM, Patel AA, Romney M-LS, Safford MM, Schluger NW, Sengupta S, Sobieszczyk ME, Zucker JE, Asadourian PA, Bell FM, Boyd R, Cohen MF, Colquhoun MI, Colville LA, de Jonge JH, Dershowitz LB, Dey SA, Eiseman KA, Girvin ZP, Goni DT, Harb AA, Herzik N, Householder S, Karaaslan LE, Lee H, Lieberman E, Ling A, Lu R, Shou AY, Sisti AC, Snow ZE, Sperring CP, Xiong Y, Zhou HW, Natarajan K, Hripsak G, Chen R (2020) Characterization and clinical course of 1000 patients with coronavirus disease 2019 in New York: retrospective case series. *BMJ* 369:m1996
  55. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, Jonigk D (2020) Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. *New England Journal of Medicine* 383:120–128
  56. Li Y, Xu Q, Ma L, Wu D, Gao J, Chen G, Li H (2020) Systematic profiling of ACE2 expression in diverse physiological and pathological conditions for COVID-19/SARS-CoV-2. *Journal of Cellular and Molecular Medicine* 24:9478–9482
  57. Li L, Qin L, Xu Z, Yin Y, Wang X, Kong B, Bai J, Lu Y, Fang Z, Song Q, Cao K, Liu D, Wang G, Xu Q, Fang X, Zhang S, Xia J, Xia J (2020) Artificial intelligence distinguishes COVID-19 from community acquired pneumonia on chest CT. *Radiology* 296: E65–E71
  58. Stelzig KE, Canepa-Escaro F, Schiliro M, Berdnikovs S, Prakash YS, Chiarella SE (2020) Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 318:L1280–L1281
  59. Song J, Li Y, Huang X, Chen Z, Li Y, Liu C, Chen Z, Duan X (2020) Systematic analysis of ACE2 and TMPRSS2 expression in salivary glands reveals underlying transmission mechanism caused by SARS-CoV-2. *Journal of Medical Virology* 92:2556–2566
  60. Bradding P, Richardson M, Hinks TSC, Howarth PH, Choy DF, Arron JR, Wenzel SE, Siddiqui S (2020) ACE2, TMPRSS2, and furin gene expression in the airways of people with asthma-implications for COVID-19. *The Journal of Allergy and Clinical Immunology* 146:208–211
  61. Vardavas CI, Nikitara K (2020) COVID-19 and smoking: a systematic review of the evidence. *Tobacco Induced Diseases* 18:20
  62. Pinto BGG, Oliveira AER, Singh Y, Jimenez L, Goncalves ANA, Ogawa RLT, Creighton R, Peron JPS, Nakaya HI (2020) ACE2 expression is increased in the lungs of patients with comorbidities associated with severe COVID-19. *The Journal of Infectious Diseases* 222:556–563
  63. Leung JM, Yang CX, Tam A, Shaipanich T, Hackett T-L, Singhera GK, Dorscheid DR, Sin DD (2020) ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. *The European Respiratory Journal* 55: 2000688
  64. Brake SJ, Barnsley K, Lu W, McAlinden KD, Eapen MS, Sohail SS (2020) Smoking upregulates angiotensin-converting enzyme-2 receptor: a potential adhesion site for novel coronavirus SARS-CoV-2 (COVID-19). *Journal of Clinical Medicine* 9:841
  65. Higham A, Singh D (2020) Increased ACE2 expression in the bronchial epithelium of COPD patients who are overweight. *Obesity* 28:1586–1589
  66. Song C, Wang Y, Li W, Hu B, Chen G, Xia P, Wang W, Li C, Diao F, Hu Z, Yang X, Yao B, Liu Y (2020) Absence of 2019 novel coronavirus in semen and testes of COVID-19 patients. *Biology of Reproduction* 103:4–6
  67. Yu Y, Xu D, Fu S, Zhang J, Yang X, Xu L, Xu J, Wu Y, Huang C, Ouyang Y, Yang L, Fang M, Xiao H, Ma J, Zhu W, Hu S, Hu Q, Ding D, Hu M, Zhu G, Xu W, Guo J, Xu J, Yuan H, Zhang B, Yu Z, Chen D, Yuan S, Shang Y (2020) Patients with COVID-19 in 19 ICUs in Wuhan, China: a cross-sectional study. *Critical Care* 24:129
  68. Levy A, Yagil Y, Bursztyn M, Barkalifa R, Scharf S, Yagil C (2008) ACE2 expression and activity are enhanced during pregnancy. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 295:R1953–R1961
  69. Bharadwaj MS, Strawn WB, Groban L, Yamaleyeva LM, Chappell MC, Horta C, Atkins K, Firmes L, Gurley SB, Brosnihan KB (2011) Angiotensin-converting enzyme 2 deficiency is associated with impaired gestational weight gain and fetal growth restriction. *Hypertension* 58:852–858
  70. Brosnihan KB, Neves LAA, Anton L, Joyner J, Valdes G, Merrill DC (2004) Enhanced expression of Ang-(1-7) during pregnancy. *Brazilian Journal of Medical and Biological Research* 37:1255–1262
  71. Baergen RN, Heller DS (2020) Placental pathology in COVID-19 positive mothers: preliminary findings. *Pediatric and Developmental Pathology* 23:177–180

72. Sutton D, Fuchs K, D'Alton M, Goffman D (2020) Universal screening for SARS-CoV-2 in women admitted for delivery. *New England Journal of Medicine* 382:2163–2164
73. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, Liao J, Yang H, Hou W, Zhang Y (2020) Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *The Lancet* 395:809–815
74. Cosma S, Carosso AR, Cusato J, Borella F, Carosso M, Bovetti M, Filippini C, D'Avolio A, Ghisetti V, Di Perri G, Benedetto C (2020) Coronavirus disease 2019 and first-trimester spontaneous abortion: a case-control study of 225 pregnant patients. *Am J Obstet Gynecol* S0002-9378(20):31177–31177
75. Marín Gabriel MA, Reyne Vergeli M, Caserío Carbonero S, Sole L, Carrizosa Molina T, Rivero Calle I, Cuadrado Pérez I, Álvarez Fernández B, Forti Buratti A, Fernández-Cañadas Morillo A, Neo-COVID-19 Research Group (2020) Maternal, perinatal and neonatal outcomes with COVID-19: a multicenter study of 242 pregnancies and their 248 infant newborns during their first month of life. *Pediatr Infect Dis J*
76. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y (2020) Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases* 94:91–95
77. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, Consortium and the NC-19 R (2020) Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA* 323:2052–2059
78. Pranata R, Lim MA, Huang I, Raharjo SB, Lukito AA (2020) Hypertension is associated with increased mortality and severity of disease in COVID-19 pneumonia: a systematic review, meta-analysis and meta-regression. *JRAAS* 21:1470320320926899–1470320320926899
79. Huang I, Lim MA, Pranata R (2020) Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia – a systematic review, meta-analysis, and meta-regression. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 14:395–403
80. Kumar A, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, Khare S, Srivastava A (2020) Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 14:535–545
81. Santoso A, Pranata R, Wibowo A, Al-Farabi MJ, Huang I, Antariksa B (2020) Cardiac injury is associated with mortality and critically ill pneumonia in COVID-19: a meta-analysis. *The American Journal of Emergency Medicine* S0735-6757(20):30280–30281
82. Pranata R, Huang I, Lim MA, Wahjoepramono EJ, July J (2020) Impact of cerebrovascular and cardiovascular diseases on mortality and severity of COVID-19—systematic review, meta-analysis, and meta-regression. *Journal of Stroke and Cerebrovascular Diseases* 29:104949
83. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, Stachel A (2020) Obesity in patients younger than 60 years is a risk factor for COVID-19 hospital admission. *Clinical Infectious Diseases* 71:896–897
84. Klang E, Kassim G, Soffer S, Freeman R, Levin MA, Reich DL (2020) Morbid obesity as an independent risk factor for COVID-19 mortality in hospitalized patients younger than 50. *Obesity* 28:1595–1599
85. Henry BM, Lippi G (2020) Chronic kidney disease is associated with severe coronavirus disease 2019 (COVID-19) infection. *International Urology and Nephrology* 52:1193–1194
86. Patanavanich R, Glantz SA (2020) Smoking is associated with COVID-19 progression: a meta-analysis. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco* 22:1653–1656
87. Alqahtani JS, Oyelade T, Aldhahir AM, Alghamdi SM, Almeahdi M, Alqahtani AS, Quaderi S, Mandal S, Hurst JR (2020) Prevalence, severity and mortality associated with COPD and smoking in patients with COVID-19: a rapid systematic review and meta-analysis. *PLOS ONE* 15:e0233147
88. Collaborative Covids (2020) Mortality and pulmonary complications in patients undergoing surgery with perioperative SARS-CoV-2 infection: an international cohort study. *Lancet* 396:27–38
89. Gómez J, Albaiceta GM, García-Clemente M, López-Larrea C, Amado-Rodríguez L, Lopez-Alonso I (2020) Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. *Gene* 762:145102
90. Yang CW, Lu LC, Chang CC, Cho CC, Hsieh WY, Tsai CH, Lin YC, Lin CS (2017) Imbalanced plasma ACE and ACE2 level in the uremic patients with cardiovascular diseases and its change during a single hemodialysis session. *Ren Fail* 39(1):719–728
91. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H (2020) Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395:1417–1418
92. Konopka KE, Nguyen T, Jentzen JM, Rayes O, Schmidt CJ, Wilson AM, Farver CF, Myers JL (2020) Diffuse alveolar damage (DAD) from coronavirus disease 2019 infection is morphologically indistinguishable from other causes of DAD. *Histopathology* 77:570–578
93. Edler C, Schröder AS, Aepfelbacher M, Fitzek A, Heinemann A, Heinrich F, Klein A, Langenwalder F, Lütgehetmann M, Meißner K, Püschel K, Schädler J, Steurer S, Mushumba H, Sperhake J-P (2020) Dying with SARS-CoV-2 infection—an autopsy study of the first consecutive 80 cases in Hamburg, Germany. *International Journal of Legal Medicine* 134:1275–1284
94. Schaefer IM, Padera RF, Solomon IH, Kanjilal S, Hammer MM, Hornick JL, Sholl LM (2020) In situ detection of SARS-CoV-2 in lungs and airways of patients with COVID-19. *Mod Pathol* 33(11):2104–2114
95. Mitchell JM, Rakheja D, Gopal P (2020) SARS-CoV-2-related hypercoagulable state leading to ischemic enteritis secondary to superior mesenteric artery thrombosis. *Clinical Gastroenterology and Hepatology* 17:S1542-3565(20)30835-1
96. Cugno M, Meroni PL, Gualtierotti R, Griffini S, Grovetti E, Torri A, Panigada M, Aliberti S, Blasi F, Tedesco F, Peyvandi F (2020) Complement activation in patients with COVID-19: a novel therapeutic target. *The Journal of Allergy and Clinical Immunology* 146:215–217
97. Ren Y, Shu T, Wu D, Mu J, Wang C, Huang M, Han Y, Zhang X-Y, Zhou W, Qiu Y, Zhou X (2020) The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. *Cellular & Molecular Immunology* 17:881–883
98. Nicklin SA (2016) A novel mechanism of action for angiotensin-(1–7) via the angiotensin type 1 receptor. *Hypertension* 68:1342–1343
99. Bao L, Deng W, Huang B, Gao H, Liu J, Ren L, Wei Q, Yu P, Xu Y, Qi F, Qu Y, Li F, Lv Q, Wang W, Xue J, Gong S, Liu M, Wang G, Wang S, Song Z, Zhao L, Liu P, Zhao L, Ye F, Wang H, Zhou W, Zhu N, Zhen W, Yu H, Zhang X, Guo L, Chen L, Wang C, Wang Y, Wang X, Xiao Y, Sun Q, Liu H, Zhu F, Ma C, Yan L, Yang M, Han J, Xu W, Tan W, Peng X, Jin Q, Wu G, Qin C (2020) The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* 583:830–833
100. Ramchand J, Patel SK, Srivastava PM, Farouque O, Burrell LM (2018) Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in

- patients with obstructive coronary artery disease. *PLOS ONE* 13: e0198144
101. Epelman S, Shrestha K, Troughton RW, Francis GS, Sen S, Klein AL, Wilson Tang WH (2009) Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. *Journal of Cardiac Failure* 15:565–571
  102. Walters TE, Kalman JM, Patel SK, Mearns M, Velkoska E, Burrell LM (2016) Angiotensin converting enzyme 2 activity and human atrial fibrillation: increased plasma angiotensin converting enzyme 2 activity is associated with atrial fibrillation and more advanced left atrial structural remodelling. *EP Europace* 19:1280–1287
  103. Ramchand J, Patel SK, Kearney LG, Matalanis G, Farouque O, Srivastava PM, Burrell LM (2020) Plasma ACE2 activity predicts mortality in aortic stenosis and is associated with severe myocardial fibrosis. *JACC: Cardiovascular Imaging* 13:655–664
  104. Li Y, Jerkic M, Slutsky AS, Zhang H (2020) Molecular mechanisms of sex bias differences in COVID-19 mortality. *Critical care* 24:405
  105. Kornilov SA, Lucas I, Jade K, Dai CL, Lovejoy JC, Magis AT (2020) Plasma levels of soluble ACE2 are associated with sex, metabolic syndrome, and its biomarkers in a large cohort, pointing to a possible mechanism for increased severity in COVID-19. *Critical care* 24:452
  106. Sama IE, Ravera A, Santema BT, van Goor H, ter Maaten JM, Cleland JGF, Rienstra M, Friedrich AW, Samani NJ, Ng LL, Dickstein K, Lang CC, Filippatos G, Anker SD, Ponikowski P, Metra M, van Veldhuisen DJ, Voors AA (2020) Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin–angiotensin–aldosterone inhibitors. *European Heart Journal* 41:1810–1817
  107. Nicin L, Abplanalp WT, Mellentin H, Kattih B, Tombor L, John D, Schmitto JD, Heineke J, Emrich F, Arsalan M, Holubec T, Walther T, Zeiher AM, Dimmeler S (2020) Cell type-specific expression of the putative SARS-CoV-2 receptor ACE2 in human hearts. *European Heart Journal* 41:1804–1806
  108. Mizuiri S, Ohashi Y (2015) ACE and ACE2 in kidney disease. *World Journal of Nephrology* 4:74–82
  109. Dudoignon E, Moreno N, Deniau B, Coutrot M, Longier R, Amiot Q, Mebazaa A, Pirracchio R, Depret F, Legrand M (2020) Activation of the renin-angiotensin-aldosterone system is associated with acute kidney injury in COVID-19. *Anaesth Crit Care Pain Med* 39(4):453–455
  110. Villard O, Morquin D, Molinari N, Raingeard I, Nagot N, Cristol JP, Jung B, Roubille C, Foulongne V, Fesler P, Lamure S, Taourel P, Konate A, Maria ATJ, Makinson A, Bertchansky I, Larcher R, Klouche K, Le Moing V, Renard E, Guilpain P (2020) The plasmatic aldosterone and c-reactive protein levels, and the severity of COVID-19: the Dyhor-19 Study. *J Clin Med* 9(7):2315
  111. Moreno-P O, Leon-Ramirez JM, Fuertes-Kenneally L, Perdiguero M, Andres M, Garcia-Navarro M, Ruiz-Torregrosa P, Boix V, Gil J, Merino E, COVID19-ALC Research Group (2020) Hypokalemia as a sensitive biomarker of disease severity and the requirement for invasive mechanical ventilation requirement in COVID-19 pneumonia: a case series of 306 Mediterranean patients. *Int J Infect Dis* 100:449–454
  112. Rieder M, Wirth L, Pollmeier L, Jeserich M, Goller I, Baldus N, Schmid B, Busch HJ, Hofmann M, Kern W, Bode C, Duerschmied D, Lother A (2020) Serum ACE-2, angiotensin II, and aldosterone levels are unchanged in patients with COVID-19. *Am J Hypertens* hpaal169
  113. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G (2020) Renin–angiotensin–aldosterone system blockers and the risk of COVID-19. *New England Journal of Medicine* 382:2431–2440
  114. Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Iturrate E, Johnson SB, Hausvater A, Newman JD, Berger JS, Bangalore S, Katz SD, Fishman GI, Kunichoff D, Chen Y, Ogedegbe G, Hochman JS (2020) Renin–angiotensin–aldosterone system inhibitors and risk of COVID-19. *New England Journal of Medicine* 382:2441–2448
  115. Fosbøl EL, Butt JH, Østergaard L, Andersson C, Selmer C, Kragholm K, Schou M, Phelps M, Gislason GH, Gerds TA, Torp-Pedersen C, Køber L (2020) Association of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use with COVID-19 diagnosis and mortality. *JAMA* 324:168–177
  116. de Abajo FJ, Rodríguez-Martín S, Lerma V, Mejía-Abril G, Aguilar M, García-Luque A, Laredo L, Laosa O, Centeno-Soto GA, Gálvez MÁ, Puerro M, González-Rojano E, Pedraza L, de Pablo I, Abad-Santos F, Rodríguez-Mañas L, Gil M, Tobías A, Rodríguez-Miguel A, Rodríguez-Puyol D, Barreira-Hernandez D, Zubiaur P, Santos-Molina E, Pintos-Sánchez E, Navares-Gómez M, Aparicio RM, García-Rosado V, Gutiérrez-Ortega C, Pérez C, Ascaso A, Elvira C (2020) Use of renin–angiotensin–aldosterone system inhibitors and risk of COVID-19 requiring admission to hospital: a case-population study. *The Lancet* 395:1705–1714
  117. Guo X, Zhu Y, Hong Y (2020) Decreased mortality of COVID-19 with renin-angiotensin-aldosterone system inhibitors therapy in patients with hypertension: a meta-analysis. *Hypertension* 76:e13–e14
  118. Gao C, Cai Y, Zhang K, Zhou L, Zhang Y, Zhang X, Li Q, Li W, Yang S, Zhao X, Zhao Y, Wang H, Liu Y, Yin Z, Zhang R, Wang R, Yang M, Hui C, Wijns W, McEvoy JW, Soliman O, Onuma Y, Serruys PW, Tao L, Li F (2020) Association of hypertension and antihypertensive treatment with COVID-19 mortality: a retrospective observational study. *European Heart Journal* 41:2058–2066
  119. Baral R, White M, Vassiliou VS (2020) Effect of renin-angiotensin-aldosterone system inhibitors in patients with COVID-19: a systematic review and meta-analysis of 28,872 patients. *Curr Atheroscler Rep* 22(10):61
  120. Milne S, Yang CX, Timens W, Bossé Y, Sin DD (2020) SARS-CoV-2 receptor ACE2 gene expression and RAAS inhibitors. *The Lancet Respiratory Medicine* 8:e50–e51
  121. Wu J, Li W, Shi X, Chen Z, Jiang B, Liu J, Wang D, Liu C, Meng Y, Cui L, Yu J, Cao H, Li L (2020) Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). *Journal of Internal Medicine* 288:128–138
  122. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, Labreuche J, Mathieu D, Pattou F, Jourdain M, group L and the LC-19 and O study (2020) High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity* 28:1195–1199
  123. Zhang F, Xiong Y, Wei Y, Hu Y, Wang F, Li G, Liu K, Du R, Wang C-Y, Zhu W (2020) Obesity predisposes to the risk of higher mortality in young COVID-19 patients. *Journal of Medical Virology* 92:2536–2542
  124. Moriconi D, Masi S, Rebelos E, Virdis A, Manca ML, de Marco S, Taddei S, Nannipieri M (2020) Obesity prolongs the hospital stay in patients affected by COVID-19, and may impact on SARS-CoV-2 shedding. *Obesity Research & Clinical Practice* 14:205–209
  125. Hajifathalian K, Kumar S, Newberry C, Shah S, Fortune B, Krisko T, Ortiz-Pujols S, Zhou XK, Dannenberg AJ, Kumar R, Sharaiha RZ (2020) Obesity is associated with worse outcomes in COVID-19: analysis of early data from New York City. *Obesity* 28:1606–1612
  126. Ryan PM, Caplice NM (2020) Is adipose tissue a reservoir for viral spread, immune activation, and cytokine amplification in coronavirus disease 2019? *Obesity* 28:1191–1194
  127. Zheng KI, Gao F, Wang X-B, Sun Q-F, Pan K-H, Wang T-Y, Ma H-L, Chen Y-P, Liu W-Y, George J, Zheng M-H (2020) Letter to the Editor: Obesity as a risk factor for greater severity of COVID-19 in patients with metabolic associated fatty liver disease. *Metabolism: Clinical and Experimental* 108:154244

128. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, Parajuli N, Penninger JM, Grant MB, Lopaschuk GD, Oudit GY (2016) ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes* 65:85–95
129. Mori J, Oudit GY, Lopaschuk GD (2020) SARS-CoV-2 perturbs the renin-angiotensin system and energy metabolism. *American Journal of Physiology-Endocrinology and Metabolism* 319:E43–E47
130. Kruglikov IL, Scherer PE (2020) The role of adipocytes and adipocyte-like cells in the severity of COVID-19 infections. *Obesity* 28:1187–1190
131. Battle D, Wysocki J, Satchell K (2020) Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clinical Science* 134:543–545
132. Yi C, Sun X, Ye J, Ding L, Liu M, Yang Z, Lu X, Zhang Y, Ma L, Gu W, Qu A, Xu J, Shi Z, Ling Z, Sun B (2020) Key residues of the receptor binding motif in the spike protein of SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. *Cellular & Molecular Immunology* 17:621–630
133. Wysocki J, Ye M, Rodriguez E, González-Pacheco FR, Barrios C, Evora K, Schuster M, Loibner H, Brosnihan KB, Ferrario CM, Penninger JM, Battle D (2010) Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: prevention of angiotensin II-dependent hypertension. *Hypertension* 55:90–98
134. Ju B, Zhang Q, Ge J, Wang R, Sun J, Ge X, Yu J, Shan S, Zhou B, Song S, Tang X, Yu J, Lan J, Yuan J, Wang H, Zhao J, Zhang S, Wang Y, Shi X, Liu L, Zhao J, Wang X, Zhang Z, Zhang L (2020) Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* 584:115–119
135. Hurlburt NK, Wan YH, Stuart AB, Feng J, McGuire AT, Stamatatos L, Pancera M (2020) Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. *Nat Commun* 11:5413
136. Wu Y, Wang F, Shen C, Peng W, Li D, Zhao C, Li Z, Li S, Bi Y, Yang Y, Gong Y, Xiao H, Fan Z, Tan S, Wu G, Tan W, Lu X, Fan C, Wang Q, Liu Y, Zhang C, Qi J, Gao GF, Gao F, Liu L (2020) A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science* 368:1274–1278
137. Shi R, Shan C, Duan X, Chen Z, Liu P, Song J, Song T, Bi X, Han C, Wu L, Gao G, Hu X, Zhang Y, Tong Z, Huang W, Liu WJ, Wu G, Zhang B, Wang L, Qi J, Feng H, Wang F, Wang Q, Gao GF, Yuan Z, Yan J (2020) A human neutralizing antibody targets the receptor binding site of SARS-CoV-2. *Nature* 584:120–124
138. Zost SJ, Gilchuk P, Case JB, Binshtein E, Chen RE, Nkolola JP, Schäfer A, Reidy JX, Trivette A, Nargi RS, Sutton RE, Suryadevara N, Martinez DR, Williamson LE, Chen EC, Jones T, Day S, Myers L, Hassan AO, Kafai NM, Winkler ES, Fox JM, Shrihari S, Mueller BK, Meiler J, Chandrashekar A, Mercado NB, Steinhardt JJ, Ren K, Loo YM, Kallewaard NL, McCune BT, Keeler SP, Holtzman MJ, Barouch DH, Gralinski LE, Baric RS, Thackray LB, Diamond MS, Carnahan RH, Crowe JE Jr (2020) Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* 584(7821):443–449
139. Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He WT, Limbo O, Smith C, Song G, Woehl J, Yang L, Abbott RK, Callaghan S, Garcia E, Hurtado J, Parren M, Peng L, Ramirez S, Ricketts J, Ricciardi MJ, Rawlings SA, Wu NC, Yuan M, Smith DM, Nemazee D, Teijaro JR, Voss JE, Wilson IA, Andrabi R, Briney B, Landais E, Sok D, Jardine JG, Burton DR (2020) Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science* 369(6506):956–963
140. Baum A, Fulton BO, Wloga E, Copin R, Pascal KE, Russo V, Giordano S, Lanza K, Negron N, Ni M, Wei Y, Atwal GS, Murphy AJ, Stahl N, Yancopoulos GD, Kyratsous CA (2020) Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* 369:1014–1018
141. Hassan AO, Case JB, Winkler ES, Thackray LB, Kafai NM, Bailey AL, McCune BT, Fox JM, Chen RE, Alsoussi WB, Turner JS, Schmitz AJ, Lei T, Shrihari S, Keeler SP, Fremont DH, Greco S, McCray PB Jr, Perlman S, Holtzman MJ, Ellebedy AH, Diamond MS (2020) A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. *Cell* 182(3):744–753.e4
142. Li W, Chen C, Drelich A, Martinez DR, Gralinski LE, Sun Z, Schäfer A, Kulkarni SS, Liu X, Leist SR, Zhelev DV, Zhang L, Kim YJ, Peterson EC, Conard A, Mellors JW, Tseng CK, Falzarano D, Baric RS, Dimitrov DS (2020) Rapid identification of a human antibody with high prophylactic and therapeutic efficacy in three animal models of SARS-CoV-2 infection. *Proc Natl Acad Sci U S A* 2:202010197
143. Miao X, Luo Y, Huang X, Lee SMY, Yuan Z, Tang Y, Chen L, Wang C, Wu F, Xu Y, Jiang W, Gao W, Song X, Yan Y, Pang T, Chen C, Zou Y, Fu W, Wan L, Gilbert-Jaramillo J, Knight M, Tan TK, Rijal P, Townsend A, Sun J, Liu X, James W, Tsun A, Xu Y (2020) A novel biparatopic hybrid antibody-ACE2 fusion that blocks SARS-CoV-2 infection: implications for therapy. *MAbs* 12(1):1804241
144. Chen P, Nirula A, Heller B, Gottlieb RL, Boscia J, Morris J, Huhn G, Cardona J, Mocherla B, Stosor V, Shawa I, Adams AC, Van Naarden J, Custer KL, Shen L, Durante M, Oakley G, Schade AE, Sabo J, Patel DR, Klekotka P, Skovronsky DM, BLAZE-1 Investigators (2020) SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with COVID-19. *N Engl J Med*
145. Li Q, Cao Z, Rahman P (2020) Genetic variability of human angiotensin-converting enzyme 2 (hACE2) among various ethnic populations. *Molecular Genetics & Genomic Medicine* 8:e1344
146. Zheng H, Cao JJ (2020) Angiotensin-converting enzyme gene polymorphism and severe lung injury in patients with coronavirus disease 2019. *The American journal of pathology* 190:2013–2017
147. Pati A, Mahto H, Padhi S, Panda AK (2020) ACE deletion allele is associated with susceptibility to SARS-CoV-2 infection and mortality rate: An epidemiological study in the Asian population. *Clinica Chimica Acta; International Journal of Clinical Chemistry* 510:455–458
148. Adamzik M, Frey U, Sixt S, Knemeyer L, Beiderlinden M, Peters J, Siffert W (2017) ACE I/D but not AGT (-6)A/G polymorphism is a risk factor for mortality in ARDS. *European Respiratory Journal* 29:482
149. Yamamoto N, Ariumi Y, Nishida N, Yamamoto R, Bauer G, Gojbori T, Shimotohno K, Mizokami M (2020) SARS-CoV-2 infections and COVID-19 mortalities strongly correlate with ACE1 I/D genotype. *Gene* 758:144944
150. Hatami N, Ahi S, Sadeghinikoo A, Foroughian M, Javdani F, Kalani N, Fereydoni M, Keshavarz P, Hosseini A (2020) Worldwide ACE (I/D) polymorphism may affect COVID-19 recovery rate: an ecological meta-regression. *Endocrine* 68:479–484
151. Mathew J, Basheeruddin K, Prabhakar S (2001) Differences in frequency of the deletion polymorphism of the angiotensin-converting enzyme gene in different ethnic groups. *Angiology* 52(6):375–379