university of groningen / faculty of science and engineering

Cytokine storm and RAAS dysregulation predispose CVD patients to severe COVID-19

Bachelor's Thesis

June 2020

Student: B. Krause
Student number: 3313883
Supervisors: Huckriede, A. & Bhide, Y.C.
Research cursus: Immunity and infectious diseases

Table of contents

Summary

The present COVID-19 pandemic is having a tremendous impact on the world, causing thousands of deaths globally. Patients with cardiovascular diseases (CVD) are considered to have an increased risk of progression to severe COVID-19 illness. A severely dysregulated immune system has been associated with disease aggravation in COVID-19. This immune system dysregulation results in a systemic hyperinflammatory state that results in acute organ injuries. Moreover, the causative virus SARS-CoV-2 causes downregulation of ACE2, which is involved in the renin-angiotensin-aldosterone-system (RAAS). ACE2 catalyzes the conversion of angiotensin II (Ang II) into Ang-(1-7) and precise balance of both systems is required for healthy regulation of essential processes (e.g. fibrosis & inflammation). Downregulation of ACE2 during SARS-CoV-2 infection results in an alteration of the Ang II/Ang-(1-7) ratio, favouring pro-inflammatory and pro-fibrotic effects of Ang II. Chronic RAAS disbalance and already existing systemic inflammation in CVD patients may predispose these patients to severe COVID-19 illness. Many CVD patients are furthermore routinely treated with RAAS inhibitors or statins, which affect ACE2 expression in tissues. Further downregulation of ACE2 upon SARS-CoV-2 infection could contribute to the RAAS dysregulation and could favour the hyperinflammatory and pro-fibrotic state in severe COVID-19.

KEY WORDS: CORONAVIRUS DISEASE 2019 • SARS-COV-2 • CYTOKINE STORM • ACE2 • RAAS

1. Introduction

As of June, 1st, the COVID-19 pandemic affected over 6.2 billion people and caused 373.883 deaths globally, with rapid growth of numbers still being reported (Dong et al., 2020). The outbreak had started early December 2019 in Wuhan City, China, with 27 patients with contagious pneumonia of unknown etiology and the number of cases quickly rose (Huang et al., 2020; Wang C. et al., 2020; Zhu et al., 2020). The causative virus appeared to be closely related to severe acute respiratory syndrome (SARS) coronavirus (CoV) and was subsequently named SARS-CoV-2 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). The lack of a vaccine or proven effective anti-viral therapy and non-existent herd immunity contributes to further dissemination of the disease. Increased knowledge about the viral infection is therefore urgently required.

SARS-CoV-2 was identified as a betacoronavirus, a coronavirus genus that primarily targets the human respiratory system (Chan et al., 2020; Letko et al., 2020). Coronaviruses are large enveloped positive sense, single-stranded RNA viruses (Lu and Liu, 2012; Woo et al., 2009). Previously identified human disease-causing coronaviruses include alphaCoVs (i.e. hCoV-229E & hCoV-NL63) and some betaCoVs (i.e. HKU1, hCoV-OC43, SARS-CoV & MERS-CoV). Whereas alphaCoVs and the betaCoVs HKU1 and hCoV-OC43 cause restricted common cold-like illnesses (Chiu et al., 2005; Gorse et al., 2009; Jean et al., 2013; Jevšnik et al., 2012), the betaCoVs SARS-CoV and MERS-CoV are known for their epidemic abilities (WHO, 2004; Zaki et al., 2012).

Viral infections start with the binding of viral particles to host receptors on the cellular surface, and receptor recognition is therefore essential for cell and tissue tropism. In coronaviruses, the membrane-bound S protein facilitates viral entry into target cells (Lu et al., 2015). Angiotensin-converting enzyme 2 (ACE2) has been identified as the major entry receptor for SARS-CoV-2 (Hoffmann et al., 2020). ACE2 is expressed in virtually all tissues, with high activities being reported in respiratory, corneal and nasal epithelial cells (Gembardt et al., 2005; Sungnak et al., 2020; Xu et al., 2020; Zhong et al., 2011).

In humans, the ACE2 receptor is involved in the renin-angiotensin-aldosterone system (RAAS), which is key player in controlling extracellular volume,

arterial pressure and tissue perfusion by regulating blood pressure and electrolyte balance (Laragh & Sealey, 1992). Unfortunately, SARS-CoV-2 infection markedly decreases ACE2 expression via binding the receptor and endocytosis of both virus and ACE2. (Zhang et al., 2020).

COVID-19 patients typically present with mild pathology, and characteristic manifestations include fever, cough and fatigue. Still, a substantial percentage of cases progresses to severe illness (Fu et al., 2020; Guan et al., 2020; Huang et al., 2020; Wang C. et al., 2020). A severely dysregulated immune system and cytokine storm are associated with disease progression to severe disease (Pedersen, 2020). Critically ill patients are often admitted to the ICU as a result of acute respiratory distress syndrome (ARDS), septic shock, acute myocardial injury and multi-organ failure, which could ultimately proceed to death (Chen et al., 2020; Goldstein et al., 2020; Huang et al., 2020, Wang C. et al., 2020).

Several studies reported that the elderly (>60 y) and patients suffering from comorbidities have a significantly higher risk of severe progression of the illness. Patients with underlying cardiovascular diseases, (e.g. hypertension, coronary artery disease and obesity) are at high risk for progression to severe COVID-19 (Lippi et al., 2020; Zhou et al., 2020). Several research groups are currently investigating how the underlying cardiovascular diseases (CVD) can predispose patients to severe COVID-19. Present insights suggest a chronic dysregulation of the RAAS system in CVD patients which may predispose them to severe COVID-19 manifestations (South et al., 2020). Many CVD patients are furthermore routinely treated with RAAS inhibitors or statins and in lieu with the fact that these treatments display a wide variety of effects in the body, treatment of these patients may play a role in this predisposition of progression to severe disease (South et al., 2020; Vaduganathan et al., 2020).

This thesis aims to address why cardiovascular patients are more prone to severe COVID-19 disease. The focus of the thesis will be on the RAAS system and dysregulated immune response implicated in severe COVID-19 disease, and the effects of cardiovascular diseases on the progression to severe COVID-19 illness.

The following research questions will be investigated:

1. What is the effect of SARS-CoV-2 infection on ACE2

2. What is the function of ACE2 within the RAAS system?

3. How does SARS-CoV-2-mediated downregulation of ACE2 affect the RAAS system?

4. How does SARS-CoV-2 infection result in the symptoms displayed by COVID-19 patients?

2. SARS-CoV-2 cell entry and life cycle

In order to understand how SARS-CoV-2 infection can result in severe COVID-19, better understanding of the viral infection is required.

The first step in viral infection is viral receptor recognition of a host cell receptor, which is mediated in coronaviruses by a viral transmembrane spike (S) protein. The S protein comprises two subunits, $S_{1,}$ which is responsible for receptor binding and $S_{2,}$ that allows fusion of viral and cellular membranes (Lee et al., 1991; Ziebuhr et al., 2001; reviewed in Fehr and Perlman, 2015).

SARS-CoV-2 cell entry is mediated by binding the ACE2 receptor, which exerts an essential role in the renin-angiotensin-aldosterone system (RAAS) (Hoffmann et al., 2020). Since virus infection is dependent on receptor recognition, ACE2 expression is the key determinant of host cell and tissue tropism. ACE2 is essentially expressed in all tissues. High expression have been reported in respiratory (i.e. alveolar epithelial cell type II), corneal and nasal endothelial cells, which are considered the main entry sites for SARS-CoV-2 (Sungnak et al., 2020; Xu et al., 2020; Zhou et al., 2020). Other high ACE2 activities are found in the heart (endothelial cells, smooth muscle cells,

cardiomyocytes), oesophagus, kidney, bladder and ileum (Gembardt et al., 2005; Xu et al., 2020; Zou et al., 2020). This expression profile is consistent with reported symptoms of COVID-19 patients (Fu et al., 2020; Wang et al., 2020; Zhu et al., 2020).

Although the exact SARS-CoV-2 cell entry remains uncertain, researchers indicate an essential role for protease cleavage (e.g. TMPRSS2) in SARS-CoV-2 cell entry and the availability of these proteases therefore contribute to host cell and tissue tropism (Sungnak et al., 2020; Hoffmann et al., 2020).

Upon cell entry, the viral RNA will allow expression of replicase gene products, which are translated into two large polypeptides (pp1a & pp1b). Processing of these polyproteins leads to 16 nonstructural proteins (nsp1 – nsp16), that form a replicase-transcriptase complex. This complex produces anti-sense genome, new viral genome and subgenomic RNA. The remaining part of the genome is translated into the structural proteins (spike (S), matrix (M), envelope (E) and nucleoprotein (N)), as well as a variable number of accessory proteins (3-5), of which some are considered to be involved in the disease pathogenesis (Frieman et al., 2007; Simmons et al., 2013). SARS-CoV, for example, contained accessory proteins that were able to interfere with gene expression through antagonizing STAT1 anti-viral activity (Frieman et al., 2007).

3.1 Classical RAAS system and COVID-19

Accumulating evidence indicates a vital role of this downregulated ACE2 in the pathophysiology of complications in severely ill COVID-19 patients (Meng et al., 2014; Uhal et al., 2011; Zheng Y., 2020). For the pathogenesis of SARS-CoV-2 infection it is crucial to understand the renin-angiotensin-aldosterone system (RAAS) system and the function of ACE2 within the RAAS system.

The RAAS system exerts an important role in controlling the homeostasis of extracellular volume, arterial pressure and tissue perfusion by regulating both blood pressure and electrolyte balance (Laragh & Sealey, 1992). The first step within the RAAS system is the release of renin by renal cells in response to a decrease in blood pressure or circulating volume (e.g. upon dehydration or ventricular pump failure) (Brown, 2006). Renin catalyzes the conversion from angiotensinogen to angiotensin I (Ang I). The enzyme angiotensin-converting enzyme (ACE) subsequently catalyzes the conversion of Ang I into the biologically active Ang II, which can bind angiotensin II type 1 (AT_1) and AT_2 receptors (Figure 1).

Most of the physiological and pathophysiological effects of Ang II is mediated via the AT_1 receptor (Figure 1). AT_1 signal transduction results in actions on the cardiovascular system (e.g. vasoconstriction, increased blood pressure, increased cardiac contractility), kidney (e.g. renal tubular sodium reabsorption, inhibition of renin release), sympathetic nervous system, and adrenal cortex (stimulation of aldosterone synthesis) (Carey & Siragy, 2003) (Figure 1).

In pathological circumstances, AT_1 signalling can results in excessive collagen deposition and enhanced inflammatory responses, cell growth, proliferation and oxidative stress, which are observed in the pathology of COVID-19 (Ferrario, 2006; Kuba et al., 2013; Patel et al., 2016; Turner et al., 2004; Zhang et al., 2020). Moreover, increased AT_1 signalling can result in vascular endothelial dysfunction, which leads to enhanced vascular permeability and subsequent (pulmonary) oedema. Severe dysregulation of Ang II could furthermore result in myocardial hypertrophy and dysfunction, cell death and arrhythmogenicity in the heart, which could ultimately result in the acute cardiac injury (Xiao et al., 2015; Intengan & Schiffrin, 2001; Montenzano et al., 2014; Boegehold et al., 2015). Acute cardiac injury is observed in some severe COVID-19 patients (Fu et al., 2020; Zhang et al., 2020).

Contrastingly, AT_2 receptor signal transduction antagonizes the effects of AT_1 signalling. Still, the exact function of the AT_2 receptor remain uncertain. The receptor is most abundant during fetal development, and AT_2 activity decreases markedly

after birth (Carey, 2003). Despite the low AT_2 expression levels, the AT_2 is thought to mediate vasodilatation, antiproliferative and apoptotic effects in adult vascular smooth muscle cells and heart (Figure 1) (AbdAlla, 2001; Carey, 2003). Still, the importance of the AT_2-mediated effects remain largely unknown.

Finally, Ang II negatively regulates ACE2 expression through the upregulation of the metalloproteinase 17 (ADAM17), that mediates the cleavage of ACE2 (Xu et al., 2017).

3.2 ACE2 and the alternative RAAS pathway

Early research on SARS-CoV-2 infection reported a downregulation of ACE2 as consequence of SARS-CoV-2 infection, with subsequent loss of catalytic activity of these receptors (Zhang, H. et al., 2020). ACE2 is part of the alternative RAAS pathway and catalyzes the conversion of Ang II into Ang-(1-7). Additionally, ACE2 converts Ang I into Ang-(1-9), and therefore prevents the conversion of Ang I into Ang II catalyzed by ACE. Ang-(1-9) can subsequently be converted to Ang-(1-7) (Roks et al., 1999). ACE2 therefore plays an important role in regulation and the balance of Ang II-mediated effects.

Ang-(1-7) binds the Mas receptor (Santos et al., 2008). Contrary to AT_1 signalling, Mas signal transduction results in vasodilatory, anti-proliferative, anti-fibrotic and anti-hypertrophic effects (Iwai & Horiuchi, 2009). Moreover, Mas signalling diminishes oxidative stress, which is involved in cell apoptosis and contributes to tissue injury (Shi et al., 2015). Mas signaling additionally alters other signaling pathways, including glucose, serum and bradykinin pathways, as well as signalling pathways activated by Ang II (Iwai & Horiuchi, 2009). Interaction of Ang-(1-7) with Mas results in upregulation of ACE2 receptor expression, thereby providing positive feedback on the alternative RAAS pathway (Iwai & Horiuchi, 2009).

Particularly important for SARS-CoV-2 infection are the effects of ACE2 and Ang-(1-7) on the inflammatory response. Ang-(1-7) has anti-inflammatory effects by diminishing Ang-II induced ROS production (Zhang et al., 2016) and downregulating the expression of pro-inflammatory molecules, such as MCP-1, VCAM-1 and IL1ß in vascular smooth muscle cells (VSMCs), which ultimately results in a reduction of cellular infiltrate (Zhang et al., 2016). This enhanced inflammatory response is generally considered to play an important role in the progression to severe COVID-19 illness (Pedersen et al., 2020; Zhang, W. et al., 2020).

Previous research further underline the organ protective effects of ACE2 in the respiratory tract. As an example, *Ace2* knockout has been implicated with severe lung disease, including enhanced vascular permeability, lung oedema, impaired lung function and increased lung fibrosis in ARDS and lung injury-mice models. Ang-(1-7) administration, on the other hand, improved oxygenation and reduced immune cell infiltrates and fibrosis in mice models. (Rey-Parra et al., 2012; Li et al., 2016). Similar organ protective effects have been reported in other organs, including heart, kidney and liver (Cheng et al., 2020). Downregulation of ACE2 upon SARS-CoV-2 infection, is likely to contribute to the symptoms (e.g. ARDS and acute organ injury) observed in COVID-19 patients.

Unfortunately, a detailed discussion of the mechanisms through which angiotensin II and angiotensin (1-7) mediate the reactions mentioned above is out of the scope of this review. The totality of evidence predominantly indicates a protective role of ACE2 in the cardiovascular, renal and respiratory systems through Mas receptor signalling and inhibition of Ang II-induced effects (Figure 1). Correct regulation of the Ang II/Ang-(1-7) ratio by ACE2 is essential in maintaining a healthy environment. Ang II and Ang-(1-7) dysregulation may contribute to detrimental effects in pathological settings as present in COVID-19.

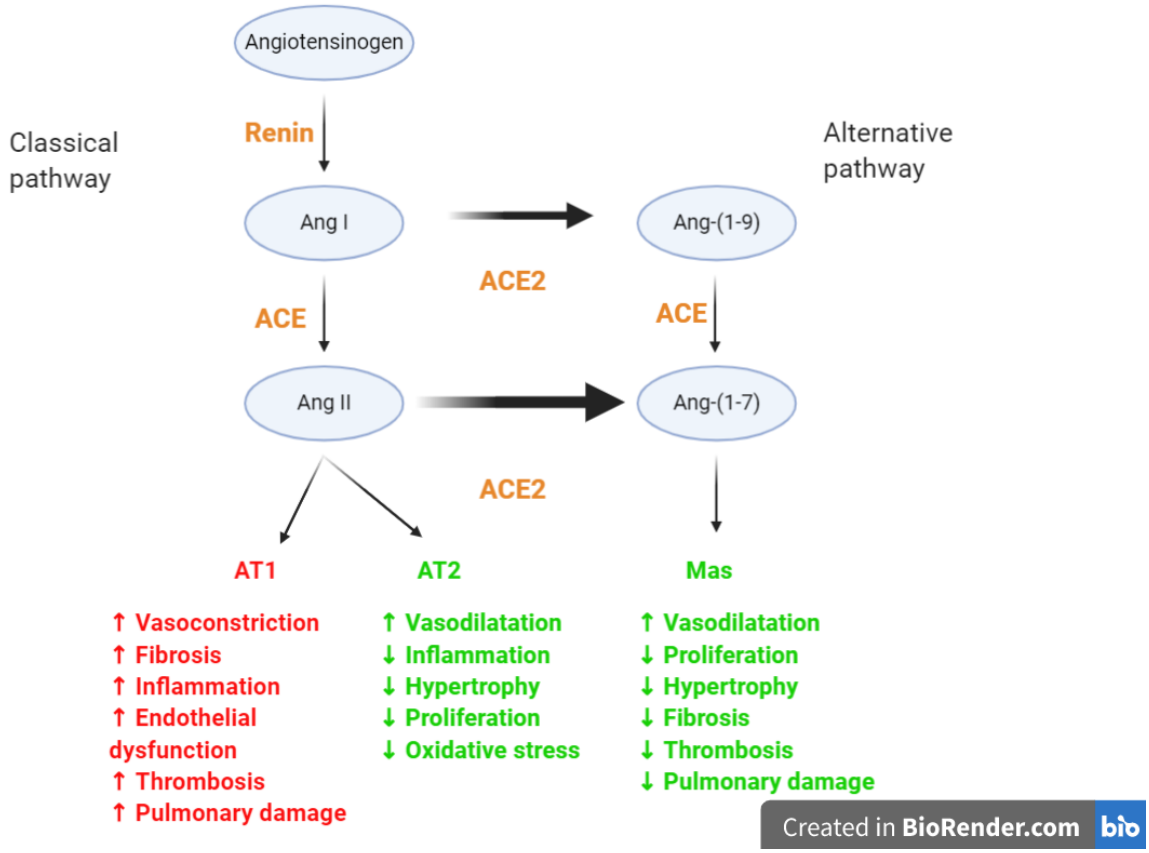

Figure 1. Renin-angiotensin-aldosterone system. *Renin converts angiotensinogen into Angiotensin I (Ang I). Ang I can be converted to Ang II by angiotensin-converting enzyme (ACE). Ang II binds AT1 and AT2 receptors. Ang I and Ang II can be converted by ACE2 to Ang-(1-9) and Ang-(1-7) respectively. Ang-(1-9) can subsequently be converted into Ang-(1-7) by ACE. Ang II and Ang-(1-7) exert counteractive effects.*

4. Impact of virus infection on ACE2 in COVID-19 patients

SARS-CoV-2 cell entry causes down-regulation of ACE2 receptors, with subsequent loss of catalytic activity of these receptors. The virus-mediated loss of ACE2 is considered to shift the system to an overall higher Ang II, and lower Ang-(1-7) tone and thereby alters the Ang II/Ang-(1-7) ratio (South et al., 2020). Laboratory findings of COVID-19 patients indeed show a significant increase in serum angiotensin II levels, which is positively correlated with viral load and lung injury (Liu et al., 2020). As a consequence of the Ang II/Ang-(1-7) alteration, Ang-(1-7) is no longer able to exert protective effects in organs, and unabated Ang II may be involved in the organ injury associated with severe COVID-19 (Liu et al., 2020). The dysregulated Ang II/Ang-(1-7) will interfere with viral infection resolution by favouring pro-inflammatory, pro-fibrotic and pro-coagulating responses.

Patients with cardiovascular disease are at higher risk of progression to severe COVID-19 illness (Lippi et al., 2020; South et al., 2020; Zhou et al., 2020). Dysregulation of the RAAS system, as well as inappropriately high Ang II is involved in many cardiovascular disorders, including hypertension, diabetes mellitus, vascular disease, atherosclerosis, infarction and heart failure (Gavras & Gavras, 2002; Atlas, 2007; Iwai & Horiuchi, 2009).

Moreover, cardiovascular patients are often routinely treated with RAAS inhibitors (e.g. ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs)). Experimental evidence revealed the ability of ACEi and ARBs to increase ACE2 receptor expression (Ferrario et al., 2005; Karram et al., 2005; Ishiyama et al., 2004). As an example, ARBs (e.g. Olmesartan) have been reported to increase both plasma concentration of Ang-(1-7) and cardiac ACE2 expression levels (Ishiyama et al., 2004; Iwata et al., 2010). Administration of ACEi similarly leads to increased cardiac ACE2 mRNA, protein and activity (Ocaranza et al., 2006; Ferrario et al., 2005). Treatment-induced upregulation of ACE2 has been hypothesized to increase the risk for SARS-CoV-2 infection and severe COVID-19 illness (South et al., 2020). However, in literature, uncertainty remains about the effects of statin and ACEi treatment during COVID-19, as some research only reports minimal effect of these treatments (Iwata et al., 2011; South et al., 2020). As increased ACE2 expression might increase the susceptibility to SARS-CoV-2 expression, intake of these drugs might predispose patients to the (cardiac) infection of SARS-CoV-2. On the other hand, since an increase of ACE2 exerts organ protective effects and contributes to repair after lung injury, one could suggest that ACE2 upregulation could prevent severe disease progression of patients on RAAS inhibitors. One paper even suggests that ACEi and ARB treatment may improve outcomes in patients with ARDS (Karram et al., 2005).

Clinical advices differed early in the COVID-19 outbreak and some physicians advices to halt ACEi/ARB treatment to avoid the potential increased risk of SARS-CoV-2 infection due to the possible ACE2 upregulation. However, in CVD patients, treatment withdrawal could be very harmful and could increase their risks of cardiovascular events. At present, since no solid evidence exist on the potential benefit or harm of these treatments, nearly all major societies have recommended against changing treatment protocols in CVD patients, unless done on clinical reasons independently of COVID-19 (Clerkin et al., 2020).

In summary, the present insights demonstrate the ability of ACEIs/ARBs to increase the expression and activity of ACE2 in heart and lung tissue performing the protective role in these systems. However, influence of ACEIs/ARBs on ACE2 expression outside these organs, as well as, whether these treatments could contribute to the degree of infection, remains uncertain.

5. Characterizing mild and severe COVID-19 patients

SARS-CoV-2 produces an acute viral infection in humans. Since the virus predominantly gains entry through the lungs, SARS-CoV-2 infection primarily results in lung injury. The most reported symptoms of COVID-19 include common respiratory virus infection symptoms such as fever, cough, fatigue and dyspnea. More rare symptoms include diarrhoea and vomiting (Fu et al., 2020; Zhang et al., 2020). The most common laboratory abnormalities in COVID-19 patients are lymphocytopenia and elevated levels of C-reactive protein and increased lactate dehydrogenase (LDH) (Fu et al., 2020). COVID-19 involves many organs and tissues in the human body (Fu et al., 2020). In one study, 36.4% of the patients presented with neurological manifestations, suggesting the involvement of the nervous system (Mao et al., 2020). Additionally, the involvement of eye (ocular surface infection), heart (arrhythmia, acute heart injury), kidney (impaired renal function), liver (abnormal liver function, microvesicular steatosis), and intestines have been reported in COVID-19 patients (Huang et al., 2020; Li et al., 2020; Xiao et al., 2020; Wang L. et al., 2020).

Although most patients present with mild symptoms, a substantial percentage of patients progress to severe COVID-19 illness (Fu et al., 2020). Already early in the pandemic, a severely dysregulated immune system, or cytokine storm was proposed to be associated with this progression to severe disease (Mehta et al., 2020;

Ye et al., 2020; Thevarajan et al., 2020). The cytokine storm is considered to be one of the major causes of ARDS and multiple-organ failure (Chousterman & Weber, 2017) and plays a vital role in disease aggravation (Shimabukuro-Vornhagen et al., 2018).

Severely ill patients are prone to a variety of complications, of which acute respiratory distress syndrome (ARDS) is the most frequent (Fu et al., 2020). ARDS is associated with the excessive release of pro-inflammatory cytokines and recruitment of white blood cells (WBCs; granulocytes and monocytes) into the lung. ARDS patients manifest with pulmonary oedema due to enhanced pulmonary vascular permeability. Oedema can result in decreased oxygen perfusion and combined with subsequent hypoxia-induced alveolar injury, and this can ultimately result in respiratory failure. (Matute-Bello et al., 2008; Katzenstein et al., 1976). Next to this inflammatory response, viral infection in the brain could attenuate the brain respiratory center and could additionally lead to respiratory failure in COVID-19 patients (Li et al., 2020).

Next to ARDS, cardiovascular-related pathologies were second most reported COVID-19 complications (Wang C., 2020; Fu et al., 2020). COVID-19-associated myocardial injury has been proposed to result from two mechanisms. First of all, myocardial injury can result from the direct effect of SARS-CoV-2 infection on the heart, resulting in acute myocarditis. Secondly, myocardial injury can result from a similar dysregulated inflammatory response as is involved in the pathogenesis of ARDS. The latter is manifested by increased levels of pro-inflammatory cytokine interleukin-6 (IL-6), LDH and D-dimer (Clerkin et al., 2020). Other organ injuries, such as renal insufficiency, is also thought to be caused by a detrimental dysregulated immune response. In a substantial part of the patients, these complications can progress to multi-organ failure or septic shock, which could ultimately result in death (Chen et al., 2020; Huang et al., 2020; Wang et al., 2020). To sum up, ARDS and cardiovascular pathologies are the most common complications of severe COVID-19 illness. A severely dysregulated immune response is thought to play an important role in the organ injuries observed in severe COVID-19 patients.

6. Cytokine storm in severe COVID-19

A severely dysregulated immune response, or cytokine storm, refers to an excessive and uncontrolled release of pro-inflammatory cytokines. The cytokine storm usually originates from the focal infected area (i.e. lungs in SARS-CoV-2 infection) and subsequently spreads via the blood circulation to other organs (Tisoncik et al., 2012). Research investigating SARS and MERS pathology showed that massive inflammatory cell infiltration and cytotoxic cytokine production could result in acute lung injury, acute respiratory distress syndrome (Figure 2) (ARDS) and death (Channappanavar & Perlman, 2017) and accumulating evidence indicates that the cytokine profile in severe COVID-19 patients resembles the cytokine storm in SARS and MERS. During SARS-CoV infection, IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGFβ, and chemokines (CCL2, 3, 5 & CXCL8-10) were associated with ARDS, whereas during MERS IL-6, IFN-α, and CCL5, CXCL8, CXCL-10 were significantly upregulated in severe patients compared to mild patients (Cameron, 2008; Channappannavar & Perlman, 2017; Fu et al., 2020; Mehta et al., 2020; Min et al., 2016; Rockx et al., 2009; Thevarajan et al., 2020; Totura & Baric, 2012; ; Ye et al., 2020).

Laboratory examination of severe COVID-19 patients revealed similar increased pro-inflammatory cytokine expression in severe patients compared to mild patients, including the cytokines IL-1B, IL-2, IL-6, IL-7, IL-8, IL-10, G-CSF (granulocyte-colony stimulating factor), IP10 (interferon-gamma-inducible protein), MCP1 (monocyte chemoattractant protein), MIP1A (macrophage inflammatory protein 1 alpha) and TNFα (tumour necrosis factor-alpha) (Wan et al., 2020; Conti et al., 2020; Chen et al., 2020; Wu & Yang., 2020; Pedersen et al., 2020; Huang et al., 2020; Wang et al., 2020; Thevarajan et al., 2020).

The pro-inflammatory cytokines IL-1ß, IL-6 and TNFα are involved in initiating the inflammatory response and are responsible for acute phase

signalling, immune cell recruitment to the site of infection, epithelial cell activation and secondary cytokine production by activated immune cells. They are furthermore responsible for causing systemic inflammatory symptoms (e.g. fever). Acute phase signalling in response to viral infection results in local effects and systemic alterations. The chemokines IL-8 and IP-10 are considered to recruit more immune infiltrates (Wu & Yang, 2020). Furthermore, high levels of IL-1B, IFN-γ, IP-10 and MCP-1 may activate T-helper type 1 (Th1) cell response, which is a crucial player in activating the specific immunity (Huang et al., 2020; Marchingo et al., 2020). Moreover, some cytokines (e.g. IL-1β, TNFα) mediate the activation of Th17 cell type responses and result in Th17 cytokine secretion (e.g. IL-17). These Th17 cytokines are considered to contribute to the cytokine storm in pulmonary infection and the formation of oedema, as seen in SARS-CoV-2 infection (Figure 2) (Tse et al., 2004; Wu & Yang, 2020). Additionally, the production of IL-10 indicates an additional involvement of activated Th2 cells (Zhu J., 2010) (Figure 2).

Contrastingly, a significant decrease in anti-viral cytokine IFN-γ expression in CD4+ T-cells has been observed in severely ill patients (Pedersen et al., 2020). IFN-γ is usually involved in inflammation resolution through repression of IL1B and inhibition of neutrophil recruitment to the site of inflammation (Blazek et al., 2015). In the end, the cytokine storm is considered responsible for a violent attack by the immune system to the body.

Regarding the cellular immune response, compared to mild patients, severe patients show an increase in white blood cells (WBC), mainly macrophages (Chen et al., 2020, Zhang et al., 2020) and increased WBC recruitment from circulation to the lungs (Figure 2) (Liao et al., 2020). Moreover, a significant reduction and exhaustion of surviving NK cells has been reported in severe patients compared to mild patients. Regarding the adaptive cellular immunity, severe patients more frequently present with a significant reduction and exhaustion of T-lymphocytes (CD4+ and CD8+) (Diao et al., 2020; Zheng et al. 2020). Finally, critical patients show characteristics of an impaired immune system revealed by splenic atrophy and lymph nodes, along with reduced lymphocytes in lymphoid organs (Zhang et al., 2020). These findings further underline a severely impaired immune regulation in COVID-19 disease.

In summary, excessive local release of cytokines, in combination with a reduced functioning cellular immune response is considered the decisive factor responsible for the pathological change and clinical manifestations observed in severe COVID-19 (Figure 2).

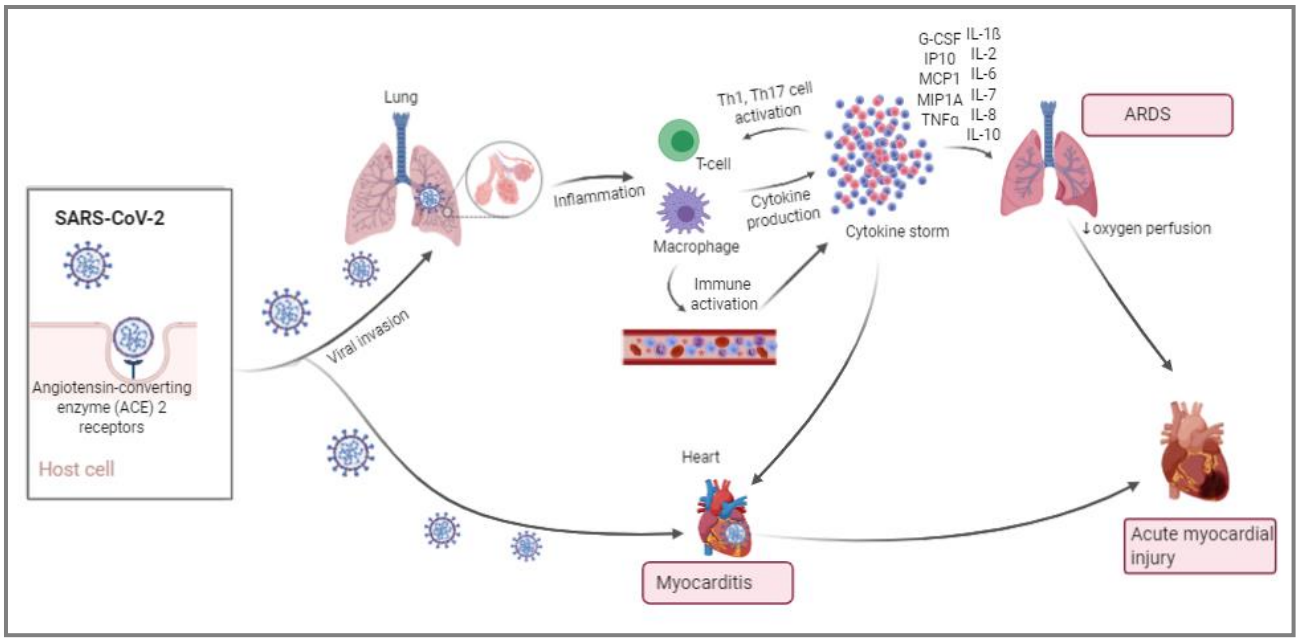

***Figure 2, proposed immune response during SARS-CoV-2 infection**. Uptake of SARS-CoV-2 in the lung results in inflammation. Activation of immune cells leads to excessive production of pro-inflammatory cytokines. Cytokine storm spread via circulation results in other organ injuries (e.g. acute myocardial injury). The hyperinflammatory response ultimately results in acute respiratory distress syndrome (ARDS) and acute myocardial injury. The figure is made with Biorender (https://biorender.com/)*

7. Discussion/conclusion

In conclusion, the dysregulated RAAS in combination with chronic inflammation in patients with underlying CVD may predispose these patients to severe COVID-19 infection.

Local excessive release of cytokines, in combination with a reduced functioning cellular immune response, is considered the decisive factor responsible for the pathological change and clinical manifestations in COVID-19 disease. This uncontrolled immune response is considered to result in ARDS and multiple organ failure, and could ultimately result in death in severe cases of SARS-CoV-2 infection. Interestingly, not all researches reported the same cytokines involved in the disease and therefore different immune involvement has been proposed by these studies.

Most studies acknowledge an essential role of IL-6 and TNFα within the cytokine storm and their levels have been correlated with ARDS severity and outcome. However, treatments targeting IL-6 show mixed results (Jamilloux et al., 2020). Experimental studies on anti-IL-6 treatment report either a suppression or facilitation of viral replication (Velazquez-Salinas et al., 2019). Effective IL-6 inhibiting treatment is probably timing dependent (Velazquez-Salinas et al., 2019). In contrast, conflicting results about IFN-γ levels in severe COVID-19 patients have been reported. Some studies showed an elevation, whereas others reported decreased IFN-γ levels (Chen et al., 2020). Increased knowledge about the role of IFN-γ in COVID-19 could contribute to potential treatments for severe COVID-19 patients.

Moreover, in most severe COVID-19 patients, a significant decrease in both CD4+ and CD8+ T-lymphocytes is observed (Chen et al., 2020; Pedersen et al., 2020; Diao et al., 2020). Remarkably, studies focusing on the cytokine storm in COVID-19 indicate an activation of both cell types, which would generally lead to the proliferation of these T-cells. A possible explanation is that virus infection results in T-lymphocyte suppression. It has been suggested that virus infection may dampen the anti-viral IFN response (Deng et al., 2020). More research into how virus infection results in this immunosuppression is required to understand the cytokine storm involved in COVID-19.

Alteration of the Ang II/Ang-(1-7) ratio in CVD patients is likely to contribute to an enhanced inflammatory response, resulting in the cytokine storm seen in severe COVID-19 patients. It should be noted that the organ protective functions of Ang-(1-7) depend on Mas receptor expression and ACE2 substrates, which could differ among tissue and cell types. As an example, Mas expression is abundant in mice brain and testis, whereas only low levels of Mas expression is observed in other tissues, including heart, kidney and lung (Villar & Pedersen, 1994; Metzger et al., 1995). This low pulmonary and cardiac expression could indicate a minor role of Ang-(1-7)/Mas signalling in COVID-19 pathogenesis. Moreover, studies researching ACE2 overexpression reveal that ACE2 does not only have organ protective functions. ACE2 overexpression in transgenic mice has for example been associated with cardiac arrhythmias (Donoghue et al., 2003) and fibrosis in spontaneously hypertensive rats (Masson et al., 2009).

As mentioned, Ang II negatively regulates ACE2 expression by upregulation of the protease ADAM17, which is responsible for ACE2 cleavage (Xu et al., 2017). If patients have increased plasma Ang II levels, one could consider that Ang II-mediated downregulation of ACE2 would make a person less susceptible to SARS-CoV-2 infection. However, it should be noted that a direct connection between ACE2 expression and the susceptibility or severity of SARS-CoV-2 infection has not been proved. Cardiovascular tissues or cells expressing ACE2 are potentially at risk for SARS-CoV-2 infection; however, other factors including expression of the host proteases that prime the virus are required for infection as well (South et al., 2020).

Next to Ang II, other substrates of ACE2 have been discovered, including physiologically active peptides such as des-Arg^{9}-bradykinin, apelin-13, dynorphin A (1–13), and β-casomorphin (Vickers et al., 2002). Downregulation of ACE2 during SARS-CoV-2 infection is likely to affect these pathways too and could be contributing to the pathology of COVID-19. However, the extent to which these pathways are altered and contribute to COVID-19 illness remains to be investigated. Functions of ACE2 may, therefore, be more complicated than initially believed. It should be noted that the outlined RAAS system in this thesis is a simplified model of a highly advanced and complex mechanism. Regarding the functions of ACE2 within the RAAS system, the RAAS system certainly contributes to COVID-19 pathology. Further research into the role of the RAAS system in COVID-19 is required and could potentially contribute to novel therapeutic targets in severely ill patients.

Evidence exists that certain ACEi and ARBs increase ACE2 expression. However, there are inconsistencies in the data. Not all studies report a similar increase, and some even indicate no effects of the same drugs. Even if ACEi and ARBs would affect ACE2 expression, it is not sure whether ACE2 expression would increase the risk for SARS-CoV-2 infection. Moreover, since withdrawal of RAAS inhibitors could deteriorate cardiovascular disease, physicians should be cautious with drug alterations. Some cardiologists suggest that patients should discontinue ACEIs/ARBs to avoid the potential increased risk of SARS-CoV-2 infection, at this time, nearly all major societies have recommended against adding or stopping ACEi, ARBs, or other RAAS antagonists unless done on clinical grounds independently of COVID-19, given the lack of evidence available on their potential benefit or harm.

Finally, the downregulation of ACE2 upon SARS-CoV-2 infection contributes to further dysregulation of the RAAS system in cardiovascular patients. This dysregulated RAAS system combined with a severely dysregulated immune system might predispose CVD patients to severe COVID-19 illness. Still, uncertainty remains about the exact RAAS and immune system involvement. Increased knowledge about these pathways is required and would potentially contribute to novel therapeutic targets in severe COVID-19 disease.

8. References

AbdAlla, S., Lother, H., Abdel-tawab, A. M., & Quitterer, U. (2001). The angiotensin II AT2 receptor is an AT1 receptor antagonist. The Journal of biological chemistry, 276(43), 39721–39726. https://doi.org/10.1074/jbc.M105253200

Atlas S. A. (2007). The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. Journal of managed care pharmacy : JMCP, 13(8 Suppl B), 9–20. https://doi.org/10.18553/jmcp.2007.13.s8-b.9

Blazek, K., Eames, H. L., Weiss, M., Byrne, A. J., Perocheau, D., Pease, J. E., Doyle, S., McCann, F., Williams, R. O., & Udalova, I. A. (2015). IFN-λ resolves inflammation via suppression of neutrophil infiltration and IL-1β production. The Journal of experimental medicine, 212(6), 845–853. https://doi.org/10.1084/jem.20140995

Boegehold, M. A., Drenjancevic, I., & Lombard, J. H. (2015). Salt, Angiotensin II, Superoxide, and Endothelial Function. Comprehensive Physiology, 6(1), 215–254. https://doi.org/10.1002/cphy.c150008

Brown, M. J. (2006). Direct renin inhibition-a new way of targeting the renin system. JOURNAL OF THE RENIN ANGIOTENSIN ALDOSTERONE SYSTEM, 7, S7.

Carey, R. M., & Siragy, H. M. (2003). Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. Endocrine reviews, 24(3), 261–271. https://doi.org/10.1210/er.2003-0001

Chan, J. F., Kok, K. H., Zhu, Z., Chu, H., To, K. K., Yuan, S., & Yuen, K. Y. (2020). Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging microbes & infections, 9(1), 221–236. https://doi.org/10.1080/22221751.2020.1719902

Channappanavar, R., & Perlman, S. (2017). Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Seminars in immunopathology, 39(5), 529–539. https://doi.org/10.1007/s00281-017-0629-x

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., & Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet (London, England), 395(10223), 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

Cheng, H., Wang, Y., & Wang, G. Q. (2020). Organ-protective Effect of Angiotensin-converting Enzyme 2 and its Effect on the Prognosis of COVID-19. Journal of medical virology.

Chiu, S. S., Chan, K. H., Chu, K. W., Kwan, S. W., Guan, Y., Poon, L. L., & Peiris, J. S. (2005). Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 40(12), 1721–1729. https://doi.org/10.1086/430301

Chousterman, B. G., Swirski, F. K., & Weber, G. F. (2017). Cytokine storm and sepsis disease pathogenesis. Seminars in immunopathology, 39(5), 517–528. https://doi.org/10.1007/s00281-017-0639-8

Clerkin, K. J., Fried, J. A., Raikhelkar, J., Sayer, G., Griffin, J. M., Masoumi, A., Jain, S. S., Burkhoff, D., Kumaraiah, D., Rabbani, L., Schwartz, A., & Uriel, N. (2020). COVID-19 and Cardiovascular Disease. Circulation, 141(20), 1648–1655. https://doi.org/10.1161/CIRCULATIONAHA.120.046941

Conti, P., Ronconi, G., Caraffa, A., Gallenga, C. E., Ross, R., Frydas, I., & Kritas, S. K. (2020). Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. Journal of biological regulators and homeostatic agents, 34(2), 1. Advance online publication. https://doi.org/10.23812/CONTI-E

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-544. doi:10.1038/s41564-020-0695-z

Diao, B., Wang, C., Tan, Y., Chen, X., Liu, Y., Ning, L., Chen, L., Li, M., Liu, Y., Wang, G., Yuan, Z., Feng, Z., Zhang, Y., Wu, Y., & Chen, Y. (2020). Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). Frontiers in immunology, 11, 827. https://doi.org/10.3389/fimmu.2020.00827

Dong, E., Du, H., & Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. The Lancet. Infectious diseases, 20(5), 533–534. https://doi.org/10.1016/S1473-3099(20)30120-1

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. Methods in molecular biology (Clifton, N.J.), 1282, 1–23. https://doi.org/10.1007/978-1-4939-2438-7_1

Ferrario C. M. (2006). Role of angiotensin II in cardiovascular disease therapeutic implications of more than a century of research. Journal of the renin-angiotensin-aldosterone system : JRAAS, 7(1), 3–14. https://doi.org/10.3317/jraas.2006.003

Ferrario, C. M., Jessup, J., Chappell, M. C., Averill, D. B., Brosnihan, K. B., Tallant, E. A., Diz, D. I., & Gallagher, P. E. (2005). Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation, 111(20), 2605–2610. https://doi.org/10.1161/CIRCULATIONAHA.104.510461

Frieman, M., Yount, B., Heise, M., Kopecky-Bromberg, S. A., Palese, P., & Baric, R. S. (2007). Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. Journal of virology, 81(18), 9812–9824. https://doi.org/10.1128/JVI.01012-07

Fu, L., Wang, B., Yuan, T., Chen, X., Ao, Y., Fitzpatrick, T., Li, P., Zhou, Y., Lin, Y. F., Duan, Q., Luo, G., Fan, S., Lu, Y., Feng, A., Zhan, Y., Liang, B., Cai, W., Zhang, L., Du, X., Li, L., … Zou, H. (2020). Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-

analysis. The Journal of infection, 80(6), 656–665. https://doi.org/10.1016/j.jinf.2020.03.041

Gavras, I., & Gavras, H. (2002). Angiotensin II as a cardiovascular risk factor. Journal of human hypertension, 16 Suppl 2, S2–S6. https://doi.org/10.1038/sj.jhh.1001392

Gembardt, F., Sterner-Kock, A., Imboden, H., Spalteholz, M., Reibitz, F., Schultheiss, H. P., Siems, W. E., & Walther, T. (2005). Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. Peptides, 26(7), 1270–1277. https://doi.org/10.1016/j.peptides.2005.01.009

Goldstein, M. R., Poland, G. A., & Graeber, C. W. (2020). Are certain drugs associated with enhanced mortality in COVID-19?. QJM : monthly journal of the Association of Physicians, hcaa103. Advance online publication. https://doi.org/10.1093/qjmed/hcaa103

Gorse, G. J., O'Connor, T. Z., Hall, S. L., Vitale, J. N., & Nichol, K. L. (2009). Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. The Journal of infectious diseases, 199(6), 847–857. https://doi.org/10.1086/597122

Guan, W. J., Liang, W. H., Zhao, Y., Liang, H. R., Chen, Z. S., Li, Y. M., Liu, X. Q., Chen, R. C., Tang, C. L., Wang, T., Ou, C. Q., Li, L., Chen, P. Y., Sang, L., Wang, W., Li, J. F., Li, C. C., Ou, L. M., Cheng, B., Xiong, S., … China Medical Treatment Expert Group for COVID-19 (2020). Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. The European respiratory journal, 55(5), 2000547. https://doi.org/10.1183/13993003.00547-2020

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., 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(2), 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052

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., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England), 395(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

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., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England), 395(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Intengan, H. D., & Schiffrin, E. L. (2001). Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. Hypertension (Dallas, Tex. : 1979), 38(3 Pt 2), 581–587. https://doi.org/10.1161/hy09t1.096249

Ishiyama, Y., Gallagher, P. E., Averill, D. B., Tallant, E. A., Brosnihan, K. B., & Ferrario, C. M. (2004). Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension (Dallas, Tex. : 1979), 43(5), 970–976. https://doi.org/10.1161/01.HYP.0000124667.34652.1a

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(7), 533–536. https://doi.org/10.1038/hr.2009.74

Iwata, M., Cowling, R. T., Yeo, S. J., & Greenberg, B. (2011). Targeting the ACE2-Ang-(1-7) pathway in cardiac fibroblasts to treat cardiac remodeling and heart failure. Journal of molecular and cellular cardiology, 51(4), 542–547. https://doi.org/10.1016/j.yjmcc.2010.12.003

Jean, A., Quach, C., Yung, A., & Semret, M. (2013). Severity and outcome associated with human coronavirus OC43 infections among children. The Pediatric infectious disease journal, 32(4), 325–329. https://doi.org/10.1097/INF.0b013e3182812787

Jevšnik, M., Uršič, T., Žigon, N., Lusa, L., Krivec, U., & Petrovec, M. (2012). Coronavirus infections in hospitalized pediatric patients with acute respiratory tract disease. BMC infectious diseases, 12(1), 365.

Karram, T., Abbasi, A., Keidar, S., Golomb, E., Hochberg, I., Winaver, J., Hoffman, A., & Abassi, Z. (2005). Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure. American journal of physiology. Heart and circulatory physiology, 289(4), H1351–H1358. https://doi.org/10.1152/ajpheart.01186.2004

Katzenstein, A. L., Bloor, C. M. & Leibow, A. A. Diffuse alveolar damage–the role of oxygen, shock and related factors. A review. Am J Pathol 85, 209–228 (1976).

Laragh, J. H., & Sealey, J. E. (2010). Renin–angiotensin–aldosterone system and the renal regulation of sodium, potassium, and blood pressure homeostasis. Comprehensive Physiology, 1409-1541.

Lee, H. J., Shieh, C. K., Gorbalenya, A. E., Koonin, E. V., La Monica, N., Tuler, J., Bagdzhadzhyan, A., & Lai, M. M. (1991). The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerase. Virology, 180(2), 567–582. https://doi.org/10.1016/0042-6822(91)90071-i

Letko, M., Marzi, A., & Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nature microbiology, 5(4), 562–569. https://doi.org/10.1038/s41564-020-0688-y

Li, Y. C., Bai, W. Z., & Hashikawa, T. (2020). The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. Journal of medical virology, 10.1002/jmv.25728. Advance online publication. https://doi.org/10.1002/jmv.25728

Li, Y., Zeng, Z., Cao, Y., Liu, Y., Ping, F., Liang, M., Xue, Y., Xi, C., Zhou, M., & Jiang, W. (2016). Angiotensin-converting enzyme 2 prevents lipopolysaccharide-induced rat acute lung injury via suppressing the ERK1/2 and NF-κB signaling

pathways. Scientific reports, 6, 27911. https://doi.org/10.1038/srep27911

Li, Z., Wu, M., Guo, J., Yao, J., Liao, X., Song, S., ... & Wu, X. (2020). Caution on kidney dysfunctions of 2019-nCoV patients. MedRxiv.

Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., Wang, Z., Li, J., Li, J., Feng, C., Zhang, Z., Wang, L., Peng, L., Chen, L., Qin, Y., Zhao, D., Tan, S., Yin, L., Xu, J., Zhou, C., … Liu, L. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China. Life sciences, 63(3), 364–374. https://doi.org/10.1007/s11427-020-1643-8

Lu, G., & Liu, D. (2012). SARS-like virus in the Middle East: a truly bat-related coronavirus causing human diseases. Protein & cell, 3(11), 803–805. https://doi.org/10.1007/s13238-012-2811-1

Lu, G., Wang, Q., & Gao, G. F. (2015). Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends in microbiology, 23(8), 468–478. https://doi.org/10.1016/j.tim.2015.06.003

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, e201127. Advance online publication. https://doi.org/10.1001/jamaneurol.2020.1127

Marchingo, J. M., Kan, A., Sutherland, R. M., Duffy, K. R., Wellard, C. J., Belz, G. T., Lew, A. M., Dowling, M. R., Heinzel, S., & Hodgkin, P. D. (2014). T cell signaling. Antigen affinity, costimulation, and cytokine inputs sum linearly to amplify T cell expansion. Science (New York, N.Y.), 346(6213), 1123–1127. https://doi.org/10.1126/science.1260044

Masson, R., Nicklin, S. A., Craig, M. A., McBride, M., Gilday, K., Gregorevic, P., Allen, J. M., Chamberlain, J. S., Smith, G., Graham, D., Dominiczak, A. F., Napoli, C., & Baker, A. H. (2009). Onset of experimental severe cardiac fibrosis is mediated by overexpression of Angiotensin-converting enzyme 2. Hypertension (Dallas, Tex. : 1979), 53(4), 694–700. https://doi.org/10.1161/HYPERTENSIONAHA.108.122333

Matute-Bello, G., Frevert, C. W., & Martin, T. R. (2008). Animal models of acute lung injury. American journal of physiology. Lung cellular and molecular physiology, 295(3), L379–L399. https://doi.org/10.1152/ajplung.00010.2008

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., & HLH Across Speciality Collaboration, UK (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet (London, England), 395(10229), 1033–1034. https://doi.org/10.1016/S0140-6736(20)30628-0

Meng, Y., Yu, C. H., Li, W., Li, T., Luo, W., Huang, S., Wu, P. S., Cai, S. X., & Li, X. (2014). Angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas axis protects against lung fibrosis by inhibiting the MAPK/NF-κB pathway. American journal of respiratory cell and molecular biology, 50(4), 723–736. https://doi.org/10.1165/rcmb.2012-0451OC

Metzger, R., Bader, M., Ludwig, T., Berberich, C., Bunnemann, B., & Ganten, D. (1995). Expression of the mouse and rat mas proto-oncogene in the brain and peripheral tissues. FEBS letters, 357(1), 27–32. https://doi.org/10.1016/0014-5793(94)01292-9

Min, C. K., Cheon, S., Ha, N. Y., Sohn, K. M., Kim, Y., Aigerim, A., Shin, H. M., Choi, J. Y., Inn, K. S., Kim, J. H., Moon, J. Y., Choi, M. S., Cho, N. H., & Kim, Y. S. (2016). Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Scientific reports, 6, 25359. https://doi.org/10.1038/srep25359

Montezano, A. C., Nguyen Dinh Cat, A., Rios, F. J., & Touyz, R. M. (2014). Angiotensin II and vascular injury. Current hypertension reports, 16(6), 431. https://doi.org/10.1007/s11906-014-0431-2

Ocaranza, M. P., Godoy, I., Jalil, J. E., Varas, M., Collantes, P., Pinto, M., Roman, M., Ramirez, C., Copaja, M., Diaz-Araya, G., Castro, P., & Lavandero, S. (2006). Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. Hypertension (Dallas, Tex. : 1979), 48(4), 572–578. https://doi.org/10.1161/01.HYP.0000237862.94083.45

Patel, V. B., Zhong, J. C., Grant, M. B., & Oudit, G. Y. (2016). Role of the ACE2/Angiotensin 1-7 Axis of the Renin-Angiotensin System in Heart Failure. Circulation research, 118(8), 1313–1326. https://doi.org/10.1161/CIRCRESAHA.116.307708

Pedersen, S. F., & Ho, Y. C. (2020). SARS-CoV-2: a storm is raging. The Journal of clinical investigation, 130(5), 2202–2205. https://doi.org/10.1172/JCI137647

Rey-Parra, G. J., Vadivel, A., Coltan, L., Hall, A., Eaton, F., Schuster, M., Loibner, H., Penninger, J. M., Kassiri, Z., Oudit, G. Y., & Thébaud, B. (2012). Angiotensin converting enzyme 2 abrogates bleomycin-induced lung injury. Journal of molecular medicine (Berlin, Germany), 90(6), 637–647. https://doi.org/10.1007/s00109-012-0859-2

Roks, A. J., van Geel, P. P., Pinto, Y. M., Buikema, H., Henning, R. H., de Zeeuw, D., & van Gilst, W. H. (1999). Angiotensin-(1-7) is a modulator of the human renin-angiotensin system. Hypertension (Dallas, Tex. : 1979), 34(2), 296–301. https://doi.org/10.1161/01.hyp.34.2.296

Santos, R. A., Ferreira, A. J., & Simões E Silva, A. C. (2008). Recent advances in the angiotensin-converting enzyme 2-angiotensin(1-7)-Mas axis. Experimental physiology, 93(5), 519–527. https://doi.org/10.1113/expphysiol.2008.042002

Shi, Y., Lo, C. S., Padda, R., Abdo, S., Chenier, I., Filep, J. G., Ingelfinger, J. R., Zhang, S. L., & Chan, J. S. (2015). Angiotensin-(1-7) prevents systemic hypertension, attenuates oxidative stress and tubulointerstitial fibrosis, and normalizes renal angiotensin-converting enzyme 2 and Mas receptor expression in diabetic mice. Clinical science (London, England : 1979), 128(10), 649–663. https://doi.org/10.1042/CS20140329

Shimabukuro-Vornhagen, A., Gödel, P., Subklewe, M., Stemmler, H. J., Schlößer, H. A., Schlaak, M., Kochanek, M., Böll, B., & von Bergwelt-Baildon, M. S. (2018). Cytokine release syndrome. Journal for immunotherapy of cancer, 6(1), 56. https://doi.org/10.1186/s40425-018-0343-9

South, A. M., Diz, D. I., & Chappell, M. C. (2020). COVID-19, ACE2, and the cardiovascular consequences. American journal of physiology. Heart and circulatory physiology, 318(5), H1084–H1090. https://doi.org/10.1152/ajpheart.00217.2020

Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., Talavera-López, C., Maatz, H., Reichart, D., Sampaziotis, F., Worlock, K. B., Yoshida, M., Barnes, J. L., & HCA Lung Biological Network (2020). SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nature medicine, 26(5), 681–687. https://doi.org/10.1038/s41591-020-0868-6

Thevarajan, I., Nguyen, T., Koutsakos, M., Druce, J., Caly, L., van de Sandt, C. E., Jia, X., Nicholson, S., Catton, M., Cowie, B., Tong, S., Lewin, S. R., & Kedzierska, K. (2020). Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nature medicine, 26(4), 453–455. https://doi.org/10.1038/s41591-020-0819-2

Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R., & Katze, M. G. (2012). Into the eye of the cytokine storm. Microbiology and molecular biology reviews : MMBR, 76(1), 16–32. https://doi.org/10.1128/MMBR.05015-11

Turner, A. J., Hiscox, J. A., & Hooper, N. M. (2004). ACE2: from vasopeptidase to SARS virus receptor. Trends in pharmacological sciences, 25(6), 291–294. https://doi.org/10.1016/j.tips.2004.04.001

Uhal, B. D., Li, X., Xue, A., Gao, X., & Abdul-Hafez, A. (2011). Regulation of alveolar epithelial cell survival by the ACE-2/angiotensin 1-7/Mas axis. American journal of physiology. Lung cellular and molecular physiology, 301(3), L269–L274. https://doi.org/10.1152/ajplung.00222.2010

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J., Pfeffer, M. A., & Solomon, S. D. (2020). Renin-Angiotensin-Aldosterone System Inhibitors in Patients with Covid-19. The New England journal of medicine, 382(17), 1653–1659. https://doi.org/10.1056/NEJMsr2005760

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. (2002). Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. The Journal of biological chemistry, 277(17), 14838–14843. https://doi.org/10.1074/jbc.M200581200

Villar, A. J., & Pedersen, R. A. (1994). Parental imprinting of the Mas protooncogene in mouse. Nature genetics, 8(4), 373–379. https://doi.org/10.1038/ng1294-373

Wan, S., Yi, Q., Fan, S., Lv, J., Zhang, X., Guo, L., Lang, C., Xiao, Q., Xiao, K., Yi, Z., Qiang, M., Xiang, J., Zhang, B., Chen, Y., & Gao, C. (2020). Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. British journal of haematology, 189(3), 428–437. https://doi.org/10.1111/bjh.16659

Wang, C., Horby, P. W., Hayden, F. G., & Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. Lancet (London, England), 395(10223), 470–473. https://doi.org/10.1016/S0140-6736(20)30185-9

Wang, L., Wang, Y., Ye, D., & Liu, Q. (2020). Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. International journal of antimicrobial agents, 105948. Advance online publication. https://doi.org/10.1016/j.ijantimicag.2020.105948

WHO (2004). Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003

Woo, P. C., Lau, S. K., Huang, Y., & Yuen, K. Y. (2009). Coronavirus diversity, phylogeny and interspecies jumping. Experimental biology and medicine (Maywood, N.J.), 234(10), 1117–1127. https://doi.org/10.3181/0903-MR-94

Wu, D., & Yang, X. O. (2020). TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi, S1684-1182(20)30065-7. Advance online publication. https://doi.org/10.1016/j.jmii.2020.03.005

Wu, D., & Yang, X. O. (2020). TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi, S1684-1182(20)30065-7. Advance online publication. https://doi.org/10.1016/j.jmii.2020.03.005

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., & Shan, H. (2020). Evidence for Gastrointestinal Infection of SARS-CoV-2. Gastroenterology, 158(6), 1831–1833.e3. https://doi.org/10.1053/j.gastro.2020.02.055

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(1), 8. https://doi.org/10.1038/s41368-020-0074-x

Xu, J., Sriramula, S., Xia, H., Moreno-Walton, L., Culicchia, F., Domenig, O., Poglitsch, M., & Lazartigues, E. (2017). Clinical Relevance and Role of Neuronal AT1 Receptors in ADAM17-Mediated ACE2 Shedding in Neurogenic Hypertension. Circulation research, 121(1), 43–55. https://doi.org/10.1161/CIRCRESAHA.116.310509

Ye, Q., Wang, B., & Mao, J. (2020). The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. The Journal of infection, 80(6), 607–613. https://doi.org/10.1016/j.jinf.2020.03.037

Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., & Fouchier, R. A. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England journal of medicine, 367(19), 1814–1820. https://doi.org/10.1056/NEJMoa1211721

Zhang, H., Penninger, J. M., Li, Y., Zhong, N., & Slutsky, A. S. (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-

2 receptor: molecular mechanisms and potential therapeutic target. Intensive care medicine, 46(4), 586–590. https://doi.org/10.1007/s00134-020-05985-9

Zhang, W., Zhao, Y., Zhang, F., Wang, Q., Li, T., Liu, Z., Wang, J., Qin, Y., Zhang, X., Yan, X., Zeng, X., & Zhang, S. (2020). The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clinical immunology (Orlando, Fla.), 214, 108393. https://doi.org/10.1016/j.clim.2020.108393

Zheng, Y. Y., Ma, Y. T., Zhang, J. Y., & Xie, X. (2020). COVID-19 and the cardiovascular system. Nature reviews. Cardiology, 17(5), 259–260. https://doi.org/10.1038/s41569-020-0360-5

Zhong, J. C., Ye, J. Y., Jin, H. Y., Yu, X., Yu, H. M., Zhu, D. L., Gao, P. J., Huang, D. Y., Shuster, M., Loibner, H., Guo, J. M., Yu, X. Y., Xiao, B. X., Gong, Z. H., Penninger, J. M., & Oudit, G. Y. (2011). Telmisartan attenuates aortic hypertrophy in hypertensive rats by the modulation of ACE2 and profilin-1 expression. Regulatory peptides, 166(1-3), 90–97. https://doi.org/10.1016/j.regpep.2010.09.005

Zhu, J., Yamane, H., & Paul, W. E. (2010). Differentiation of effector CD4 T cell populations (*). Annual review of immunology, 28, 445–489. https://doi.org/10.1146/annurev-immunol-030409-101212

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G. F., Tan, W., & China Novel Coronavirus Investigating and Research Team (2020). A Novel Coronavirus from Patients with Pneumonia in China, 2019. The New England journal of medicine, 382(8), 727–733. https://doi.org/10.1056/NEJMoa2001017

Ziebuhr, J., Thiel, V., & Gorbalenya, A. E. (2001). The autocatalytic release of a putative RNA virus transcription factor from its polyprotein precursor involves two paralogous papain-like proteases that cleave the same peptide bond. The Journal of biological chemistry, 276(35), 33220–33232. https://doi.org/10.1074/jbc.M104097200

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(2), 185–192. https://doi.org/10.1007/s11684-020-0754-0