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PATHOPHYSIOLOGY OF CRITICALLY ILL COVID-19 PATIENTS

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PATHOPHYSIOLOGY IN CRITICALLY ILL COVID-19 PATIENTS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To all COVID-19 patients

"Knowledge is an unending adventure at the edge of uncertainty."

Jacob Bronowski

POPULAR SCIENCE SUMMARY OF THE THESIS

With the onset of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a new disease, COVID-19, challenged health care professionals and scientists. The characteristics of the disease differed in several important aspects from our previous experiences of viral pneumonia and the search for the underlying pathophysiology became imperative to find preventive measures and to provide optimal care.

The receptor used by the virus for cell entry, the angiotensin converting enzyme type 2 (ACE2), is part of a central hormonal system, the renin-angiotensin-aldosterone system (RAAS). This system is involved in several critical physiological processes of which the regulation of blood pressure as well as water-and electrolyte balance are those most commonly described. The ACE2 is a crucial component to maintain hormonal balance within this system and this balance is important for health. Consequently, a disruption of this hormonal system by viral interference with the ACE2 has been proposed as major pathway in the development of severe COVID-19. In diseases such as hypertension, diabetes, or obesity, elevated ACE2 in the blood indicates an pre-existing underlying chronic RAAS-imbalance. These diseases are now known risk factors for developing severe COVID-19.

The studies in this thesis investigated the importance of SARS-CoV-2 interference in this hormonal system for the development of severe COVID-19.

The first study was a so-called translational study in which the findings from an experimental model were compared with clinical data from patients. In this study, we performed a large-animal experiment where a pharmacological imbalance of the RAAS, like the one described in COVID-19, was investigated. The clinical findings from this experiment were then compared with data from COVID-19 patients. The conclusion was that when manipulating the RAAS in a similar manner to the one described to occur in viral interaction, a COVID-19-like state was created. This finding indicates that imbalance in the RAAS may in part contribute to severe COVID-19.

In the second study we investigated if long-term blood glucose values, using the HbA1c test, were associated with the risk of developing respiratory failure in a cohort of hospitalised SARS-CoV-2-infected patients. The conclusion was that patients with either unknown chronic elevation of blood glucose levels or poorly controlled diabetes had a markedly increased risk of developing respiratory failure.

In the third study, investigation of any association between fluctuations in electrolyte- and fluid status and poor outcome were performed in hospitalised COVID-19 patients. The conclusion from this study was that the need for mechanical ventilation or longer hospital stay or death was increased in patients with either low or high levels of sodium.

Finally, in study IV, plasma concentrations of three different hormones of the RAAS and a water regulative hormone (vasopressin) closely related to the RAAS were analysed in

COVID-19 patients in the ICU and compared to a healthy control group. The conclusion was that that ACE2 and vasopressin were low in COVID-19 patients compared to the healthy control group, indicating viral induced interference in our hormonal systems.

In summary, there are several findings in these studies indicating an association between an imbalanced RAAS and the development of severe COVID-19.

POPULÄRVETENSKAPLIG SAMMANFATTNING

I och med spridningen av coronaviruset SARS-CoV-2 följde en ny sjukdom, COVID-19. Symtomen vid COVID-19 varierar från person till person och sträcker sig från lindriga förkylningssymtom till livshotande intensivvårdskrävande lunginflammation. Den kliniska bilden vid intensivvårdskrävande COVID-19 skiljer sig på flera sätt från tidigare erfarenheter av virusorsakad lunginflammation. Således är en ökad förståelse för de underliggande sjukdomsmekanismerna vid svår COVID-19 av största vikt för bästa möjliga vård av dessa patienter såväl som för sjukdomsförebyggande insatser.

Tidigt under pandemin stod det klart att viruset SARS-CoV-2 använder sig ett specifikt protein i vår kropp för att ta sig in i cellerna och föröka sig. Detta protein, ACE2, har som huvuduppgift att upprätthålla balans inom ett våra hormonsystem, RAAS. Då detta hormonsystem är involverat i ett flertal livsviktiga fysiologiska processer, så som blodtrycksreglering och upprätthållande av normal salt-och vätskebalans, är en balans inom detta hormonsystem av största vikt för hälsa. En virusorsakad störning av detta hormonsystem genom bindningen till proteinet ACE2 har således föreslagits som orsak till svår COVID-19. Vid sjukdomar som till exempel högt blodtryck, diabetes eller övervikt, har uppmätta förhöjda nivåer av ACE2 i blodet indikerat en underliggande kronisk obalans inom RAAS. Dessa sjukdomar är numera kända riskfaktorer för att utveckla allvarlig COVID-19.

Studierna i denna avhandling undersökte betydelsen av virusets påverkan av RAAS och dess samband med utvecklandet av svår COVID-19.

Den första studien var en så kallad translationell studie där resultaten från en experimentell modell jämfördes med kliniska data från patienter. I denna studie utförde vi ett stordjurs-experiment där en läkemedelsorsakad obalans i RAAS, liknande den som beskrivits för COVID-19, undersöktes. De kliniska fynden från detta experiment jämfördes sedan med data från COVID-19 patienter. Slutsatsen var att vid en sådan manipulation av hormonsystemet RAAS kunde ett COVID-19-liknande tillstånd skapas. Detta fynd tyder på att obalans i RAAS delvis kan bidra till allvarlig COVID-19.

I den andra studien undersökte vi om kroniskt förhöjda blodsockervärden, med hjälp av HbA1c-test, hos sjukhusvårdade SARS-CoV-2-infekterade patienter påverkade risken att utveckla andningssvikt. Slutsatsen var att patienter med antingen okänd kronisk förhöjning av blodsockernivåerna eller dåligt kontrollerad diabetes hade en markant ökad risk att utveckla svår andningssvikt.

I den tredje studien undersöktes sambandet mellan vatten-och saltförändringar och behovet av respiratorbehandling eller död hos sjukhusvårdade patienter med COVID-19. Slutsatsen från denna studie var att behovet av respiratorbehandling, längre sjukhusvistelse eller dödsfall var ökat hos de patienter där saltnivåerna var antingen låga eller höga.

Slutligen, i studie IV, analyserades blodnivåer av tre olika hormoner inom RAAS samt det vattenreglerande hormonet vasopressin, vars funktion är nära kopplat till RAAS, hos

intensivvårdspatienter med svår COVID-19. En frisk kontrollgrupp användes för jämförelse. Slutsatsen var att ACE2 och vasopressin var låga hos COVID-19-patienter jämfört med den friska kontrollgruppen, vilket tyder på en virusorsakad påverkan av våra hormonella system.

Sammanfattningsvis finns det flera fynd i dessa studier som indikerar ett samband mellan ett obalanserat RAAS och utvecklingen av svår COVID-19.

ABSTRACT

With the onset of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a new disease, COVID-19, challenged health care professionals and scientists. The angiotensin-converting enzyme type 2 (ACE2) was identified early in the pandemic as being the receptor used by SARS-CoV-2 for cell entrance. Since this discovery, a disruption of the renin-angiotensin-aldosterone system (RAAS) has been established as one of the main pathophysiological pathways for developing severe COVID-19. The RAAS is closely linked to cardiovascular health and a strict balance within the system is therefore crucial. The ACE2 is key to upholding this balance by keeping angiotensin II (ANG II) levels appropriate for the physiological demand. Any interference with ACE2-activity may result in increased unwanted ANG II effects such as hypertension, insulin resistance, thrombosis, and organ failure, all frequently seen during severe COVID-19.

The overall aim of this thesis was to investigate the pathophysiological consequences of the viral interference with ACE2 and thus the RAAS, and its association with the clinical presentation of severe COVID-19.

Study I: In this translational study, we induced a pharmacological RAAS imbalance in swine, either by infusing ANG II or/and blocking ACE2. This causes a pathophysiological state (including impaired lung perfusion, increased pulmonary artery pressure, decreased oxygenation, alveolar damage, and acute kidney injury) which shared several clinical and radiological features observed in severe COVID-19.

Study II: In a retrospective observational study of 385 hospitalised SARS-CoV-2 positive patients, we investigated the association between chronic dysglycemia, diagnosed by analysis of available HbA1c, and SARS-CoV-2 associated respiratory failure. We found that prediabetes (OR 14.41 [95% CI 5.27-39.43]), unknown diabetes (OR 15.86 [95% CI 4.55-55.36]) and uncontrolled diabetes (OR 17.61 [95% CI 5.77-53.74]) were associated with an increased risk of respiratory failure, independent of age, sex, and BMI.

Study III: In this retrospective observational study of 406 hospitalised patients, we investigated the dynamics of common plasma electrolytes and acid-base biomarkers in patients with severe COVID-19 and its association with the requirement for invasive ventilation and mortality. We found hyponatremia on admission in 53% of the studied population followed by the development of hypernatremia in 42% the patients within the first 2 weeks of hospitalisation. The development of hypernatremia was associated with a more severe course of COVID-19 and increased 30-day mortality (OR 3,94 [95% CI 2,27-6.85)

Study IV: In this prospective observational study, we compared circulating concentrations of three main biomarkers of the RAAS (ANG II, ANG 1-7 and ACE2) and arginine vasopressin (copeptin) in 56 COVID-19 patients admitted to the intensive care unit. We found plasma concentration of ACE2 and copeptin to be low compared to healthy controls. These findings were not combined with low concentrations of ANG 1-7 or hypotension.

We conclude that the interaction of SARS-CoV-2 with the RAAS may explain several of the clinical findings observed in COVID-19 patients. Patients with a pre-existing RAAS-imbalance may suffer from acute on chronic RAAS-activation with acceleration of several metabolic manifestations when infected with SARS-CoV-2. The complexity of RAAS and its impact on health and sickness needs to be further investigated, expanding the arsenal of tools for earlier identification of any developing imbalance within the system as well as intensifying treatment options to prevent disease progression.

In summary, there are several findings in these studies indicating an association between an imbalanced RAAS and the development of severe COVID-19.

LIST OF SCIENTIFIC PAPERS

I. **COVID-19 pathophysiology may be driven by an imbalance in the renin-angiotensin-aldosterone system**

Susanne Rysz, Jonathan Al-Saadi, Anna Sjöström, Maria Farm, Francesca Campoccia Jalde, Michael Plattén, Helen Eriksson, Margareta Klein, Roberto Vargas-Paris, Sven Nyrén, Goran Abdula, Russell Ouellette, Tobias Granberg, Malin Jonsson Fagerlund, Johan Lundberg.
Nature Communications, 2021;12 (1): 2417

II. **Chronic dysglycemia and risk of SARS-CoV-2 associated respiratory failure in hospitalized patients.**

Susanne Rysz, Malin Jonsson Fagerlund, Claire Rimes-Stigare, Emma Larsson, Francesca Campoccia Jalde, Johan Mårtensson.
Acta Anaesthesiologica Scandinavica, 2022; 66(1):48-55.

III. **Electrolyte and acid-base imbalance in severe COVID-19**

Anna Sjöström, Susanne Rysz, Henrik Sjöström, Charlotte Höybye
Endocrine Connections, 2021; 10(7): 805–814.

IV. **Endocrine biomarkers in COVID-19, an observational study.**

Susanne Rysz, Emelie Dickman Kahm, Anna Sjöström Johan Lundberg, Anette Ebberyd, Therese Djärv, Anders Oldner*, Malin Jonsson Fagerlund*
Manuscript

* These authors contributed equally to the paper

SCIENTIFIC PAPERS BY THE AUTHOR BUT *NOT* INCLUDED IN THE THESIS

I. **The effect of levosimendan on survival and cardiac performance in an ischemic cardiac arrest model. A blinded randomized placebo-controlled study in swine**

Susanne Rysz, Johan Lundberg, Per Nordberg, Helen Eriksson, Björn Wieslander, Magnus Lundin, Alexander Fyrdahl, John Pernow, Martin Ugander, Therese Djärv, Malin Jonsson Fagerlund.
Resuscitation, 2020;150:113-120

II. **Thromboembolism, Hypercoagulopathy, and Antiphospholipid Antibodies in Critically Ill Coronavirus Disease 2019 Patients: A Before and After Study of Enhanced Anticoagulation**.

Jan van der Linden, Lou Almskog, Andreas Liliequist, Jonatan Grip, Thomas Fux, Susanne Rysz, Anna Ågren, Anders Oldner, Marcus Ståhlberg
Crit Care Explor 2020, **2**(12):e0308.

III. **Continuous renal replacement therapy in intensive care patients with COVID-19; survival and renal recovery**.

Karin E Eriksson, Francesca Campoccia-Jalde, Susanne Rysz, Claire Rimes-Stigare.
*Journal of critical care 2021, **64**:125-130.*

IV. **The use of Levosimendan after out-of-hospital cardiac arrest and its association with outcome-an observational study**

Susanne Rysz, Malin Jonsson Fagerlund, Johan Lundberg, Mattias Ringh, Jacob Hollenberg, Marcus Lindgren, Martin Jonsson, Therese Djärv, Per Nordberg.

Submitted manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ACE2	Angiotensin converting enzyme type 2
ADH	Anti-diuretic hormone
ANG 1-7	Angiotensin 1-7
ANG 1-9	Angiotensin 1-9
ANG II	Angiotensin II
ARB	Angiotensin receptor blocker
ARDS	Acute respiratory distress syndrome
AT1R	Angiotensin receptor type 1
AVP	Arginine vasopressin
BMI	Body mass index
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CTPA	Computed Tomography Pulmonary Angiography
ELISA	Enzyme-Linked Immunosorbent Assay
HbA1c	Glycated hemoglobin
ICU	Intensive Care Unit
LMWH	Low-Molecular-Weight Heparin
mACE2	Membrane bound Angiotensin Converting Enzyne type 2
MERS-CoV	Middle East respiratory syndrome coronavirus
MRI	Magnetic Resonance Imaging
OR	Odds ratio
PCR-test	Polymerase Chain Reaction test
RAAS	Renin-angiotensin-aldosterone system
sACE2	Soluble Angiotensin Converting Enzyne type 2
SARS-CoV	Severe Acute Respiratory Syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
WHO	The World Health Organization

1 INTRODUCTION

This thesis began in a completely different field of critical care medicine. Clinical observations led us to hypothesising that the inotropic-inodilating cardiac failure drug levosimedan would be of benefit during resuscitation in cardiac arrest. Thus, my original thesis aimed to investigate the effects of levosimendan in cardiac arrest. We performed a large animal model of ischemic cardiac arrest using a blinded randomized method. We demonstrated that levosimendan given intra-arrest and during the first 24-hours of post-resuscitation care improved survival and cardiac performance compared to placebo[1]. Thereafter we investigated the usage of levosimendan in out-of-hospital cardiac arrest patients admitted to the intensive care units in the Stockholm region. We plan to further explore the mechanisms behind the observed beneficial hemodynamic effects of levosimendan in cardiac arrest.

However, the project was abruptly interrupted by the onset of the COVID-19 pandemic and the thesis were temporarily put on hold in favour of face masks and protective clothing. In the bedside work with intensive care COVID-19 patients, many knowledge gaps were encountered, and these became fuel to comprehend more and to remain lucid at the time. In addition, we witnessed a new disease, in which a uniquely homogenous patient group presented with atypical symptoms that responded poorly to established treatments for viral pneumonia.

The absence of a clear pathophysiological understanding and a lack of evidence for intervention in the presence of a de novo disease where all issues that needed to be addressed to deliver optimized care and instigate preventive measures.

This thesis is the result of endless curiosity, an escalating frustration, a sense of urgency, a unifying collaboration, and an infinite respect for the potency of the smallest of living particles. Hopefully, this thesis will be another piece in in our common and yet unsolved, COVID-19-puzzle.

2 BACKGROUND.

2.1 SARS-COV-2 PANDEMIC

With the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a new disease, COVID-19, challenged health care professionals and scientists. What was initially believed to be a common viral pneumonia causing acute respiratory stress syndrome (ARDS) turned out to be something unknown and, in many aspects, different.

The coronaviruses are a large family of viruses that mostly circulate among diverse animal species, i.e., camels and bats[2]. The novel coronavirus, SARS-CoV-2, is the seventh known coronavirus to infect humans and the third one to cause serious and widespread illness and death (COVID-19)[3].

The first novel coronavirus to emerge was the coronavirus SARS-CoV, causing the disease severe acute respiratory syndrome (SARS) in 2002-2004. The second novel coronavirus to be identified was the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. Sporadic and localised outbreaks of the disease, MERS, are still occurring[4].

In December 2019, the first reports of the novel coronavirus SARS-CoV-2, causing the coronavirus disease 2019 (COVID-19), came from Wuhan, China[5]. Due to the rapid spread of the virus and the disease severity, COVID-19 was declared a global pandemic by the World Health Organization on March 11, 2020[6]. The main clinical findings seen in all these viruses are a respiratory failure. However, the higher mortality rate associated with MERS (~35%) and SARS-CoV(10-15%) compared to SARS-CoV-2 (2-4%) may be due to the different receptor used by MERS for cell entry and, perhaps improvements in health care[2, 4, 7].

In Sweden, the first confirmed infection of SARS-CoV-2 was reported from the county of Jönköping in January 2020, in a young woman who had recently visited the Wuhan region of China. Since then, about 2.5 million people have been infected and approximately 18000 deaths have been reported in Sweden so far[6].

Worldwide, over 400 million people has been infected by SARS-CoV-2 and nearly 6 million deaths has been recorded according to reports from WHO[6]. Due to large case numbers and homogeneity in characteristics and presentation, we have now acquired some understanding of the disease[8]. Several risk factors associated with the development of severe illness have thus been identified, including increased age, male sex, obesity, hypertension, and diabetes[9, 10]. There is though, still much to learn regarding the pathophysiology of COVID-19 for prevention, optimized clinical practice, and improved outcome. In addition, a better understanding of SARS-CoV-2 and its interaction in human physiology may in the long run contribute to improved public health.

2.2 SARS-COV-2 VIRUS

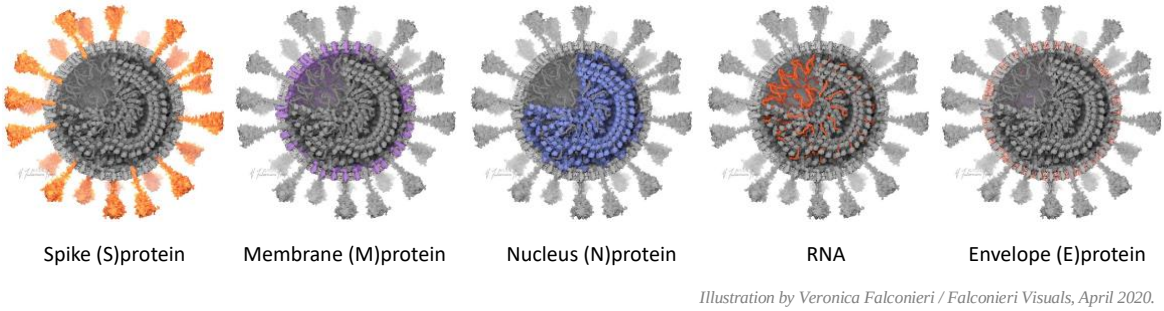

Figure 1. *Illustrations of the SARS-CoV-2 structure.*

The SARS-CoV-2 genome is a single-stranded RNA. The virus contains four structural proteins (S, E, M, and N) and 16 non-structural proteins (nsp1-16), the later primarily involved in replication, transcription, signaling, and modulation of host cell defenses (Figure 1)[3, 11-13]. The viral genome is covered by the nucleus (N) protein forming a protein shell outside the genome which is then further packed by an envelope into which the structural proteins; membrane (M) protein spike (S) protein [13], and envelope (E) protein are incorporated with different functions[3].

The surface of each corona virion is covered by protruding crown-shaped projections, the transmembrane S proteins (Figure 1). In a trimeric configuration, the S proteins are responsible for host cell receptor recognition and viral entry into host cells[11, 12]. Individual virions have been estimated to contain about 75-300 spikes, each with receptor binding capacity in a trimeric position[14]. The total number of virions at peak infection has been estimated to be around 1-100 billion virions[15], each with 75-300 S-proteins, indicating a significant receptor-binding-capacity. To enter host cells, SARS-CoV-2, like the predecessor SARS-CoV, uses the angiotensin converting enzyme type 2 (ACE2)[16, 17], a crucial hormone peptide in the renin-angiotensin-aldosterone system (RAAS). However, SARS-CoV-2 exhibits a significantly higher affinity for human ACE2 compared to SARS-CoV, something that may explain the higher infectivity observed for SARS-CoV-2[17].

2.3 THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS)

The RAAS is a hormonal system made up of several hormones, crucial for normal physiology and survival. The system plays a central role in the regulation of renal, cardiac, and vascular physiology. It comprises two opposing hormonal axes, the classical and the alternative RAAS, both strictly regulated by intrinsic and extrinsic feed-back-feed-forward inputs crucial for homeostasis[18, 19].

The classical RAAS, starts with renin and ends with the main effector peptide ANG II in response to hypotension, low sodium levels and sympathetic nerve activation (Figure 2)[19, 20].

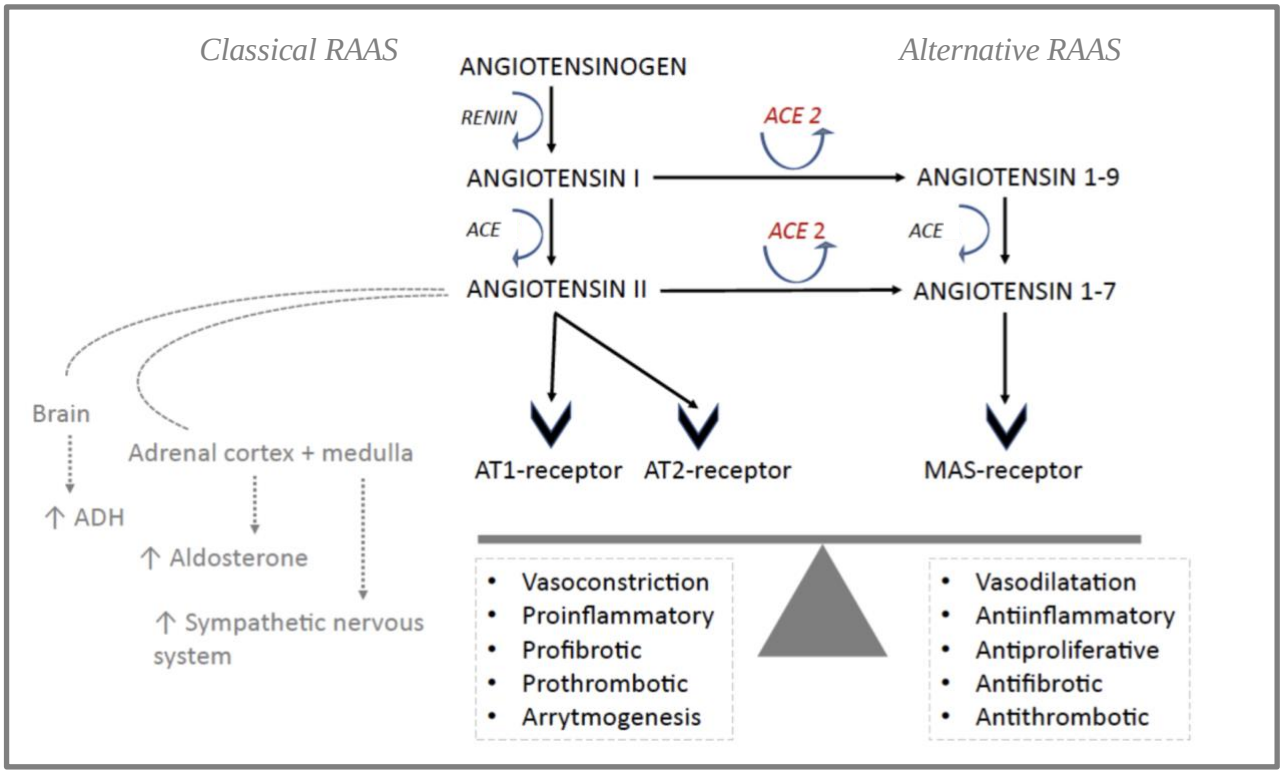

Figure 2. *The renin-angiotensin-aldosterone system (RAAS). ACE= Angiotensin Converting Enzyme; ACE 2= Angiotensin Converting Enzyme type 2; AT1 and AT2-receptor= Angiotensin II receptor type 1 and 2; ADH=Antidiuretic hormone.*

Acting on its main receptor angiotensin II receptor type 1(AT1R), the effects of classical RAAS activation are several listed in Table 1[20, 21]. In life threatening situations, for example massive bleeding or in hypovolemic shock states, a rapid elevation of ANG II aims to restore cardiovascular and renal homeostasis by increasing extracellular volume and thus perfusion, secure energy supplies to vulnerable organs, minimizing bleeding, and activate an appropriate immune response. A less pronounced chronic activation of the classical RAAS, generating a similar but dampened response, has been linked to cardiovascular and metabolic morbidity and mortality[18, 22, 23].

Being the main effector peptide of classical RAAS, ANG II, is a potent endocrine and paracrine hormone. It has a short circulatory half-life (estimated to about 15-30 seconds) before being converted to other angiotensin's or docking to its receptor[24, 25]. As with many other hormones, this short half-life complicates the analysis of circulating levels of ANG II *in vivo*.

The alternative RAAS, aims to counterbalance the classical RAAS, generating the end-product angiotensin 1-7 (ANG 1-7) as shown in Figure 2[20, 26]. The ACE2 is a key enzyme in the alternative RAAS, generating ANG 1-7 primarily by cleavage of ANG II to ANG 1-7 and to a lesser extent, by formation of angiotensin 1-9 (ANG1-9) from angiotensin I[27]. The ANG 1-9 is then converted to ANG 1-7 by ACE or different peptidases. The main receptor for ANG 1-7 is the MAS-receptor, a G-protein-coupled transmembrane receptor expressed in

the brain, heart, kidney, liver, and vascular endothelium[20, 28]. The effects resulting from ANG 1-7-MAS-receptor interaction largely oppose those of the classical RAAS and are therefore considered organ-protective (Table1). The ANG 1-7 can also be formed directly from ANG I by the enzyme neprilysin in an ACE2-independent metabolic route[28]. However, kinetic studies indicates that the most efficient pathway for ANG 1-7 formation is the ACE2 cleavage of ANG II[29, 30].

Table 1. *Physiological and clinical effects of classical and alternative RAAS[21, 31-38]. SVR=systemic vascular resistance; PAI-1=plasminogen activator inhibitor 1.*

	Angiotensin II		**Angiotensin 1-7**	
Effector organ	***Physiological effects***	***Clinical effects***	***Physiological effects***	***Clinical effects***
Vasculature	Vasoconstriction	Increased blood pressure	Vasodilatation	Lowered blood pressure and SVR
Kidneys	Na^+, Cl^-, H_2O reabsorbtion	Increased blood pressure,	Excretion of Na^+, Cl^-, H_2O	Natriuresis, diuresis
Nervous system	Sympathetic activation	Hypertension, tachycardia,	No sympathetic activation	
Lungs	Pulmonary vasoconstriction	Pulmonary hypertension	Pulmonary vasodilation	Increased pulmonary perfusion
Metabolism	Influence metabolic pathways	Insulin resistance, hyper lipidemia	Influence metabolic pathways	Improved glycemic- and lipid status
Pituitary	Vasopressin release	Hypertension, anti-diuresis, water retention	Inhibited vasopressin release	Natriuresis, diuresis.
Adrenal gland	Release of aldosterone	Na^+-absobtion, K^+-excretion, H^+-excretion	Homeostasis	Homeostasis
Coagulation	Increased release of PAI-1	Thrombotic events	Preserved fibrinolysis	Anti-trombotic
Immune system	Upregulation of cytokines, ROS-production,Immune cell activation	Inflammation, fibrosis	Balance immune response	Tissue protection

2.4 THE ANGIOTENSIN CONVERTING ENZYME TYPE 2 (ACE2)

Since the discovery of ACE2 in 2000, its important role in the RAAS has become evident[3, 27, 39]. Acting as a stabilising bridge between the classical and the alternative RAAS, ACE2 activity opposes many of ANG IIs´ hazardous effects through the conversion of ANG II to the more protective peptide ANG 1-7[20, 40]. Consequently, the alternative RAAS is now often described as the protective arm of the RAAS.

The ACE2 is a transmembrane glycoprotein (mACE2), with an extracellular catalytic domain that can be shed into the circulation in a catalytically active form (sACE2)[27]. The shedding process occurs in response to high ANG II and inflammation[41-43]. In this way, sACE2 can easily gain access to circulating ANG II for degradation and normalisation of the ANG II/ANG 1-7 ratio. The mACE2 is widely express throughout the body, e.g., the heart, kidney, endothelium, gastrointestinal tract, upper airways[39, 42, 44].

In physiological homeostasis, the levels of circulating sACE2 are low[45]. The levels of sACE2 are higher in men compared to women and described as low in children, even though expressing a high total ACE2-activity[46-48]. Conversely, in chronic over-activation of classical RAAS, increased shedding of mACE2 will occur with a subsequent increase in the levels of sACE2 for counterbalancing any increase in ANG II[42].

Increased levels of sACE2 have been found in several cardiometabolic diseases, including hypertension, diabetes mellitus, heart failure, obesity, and atherosclerosis indicating an underlying activation of classical RAAS in these diseases. Increased levels of sACE2 have also been associated with several pathological responses including insulin resistance, inflammation, thrombosis, oxidative stress, and fibrosis[49-52]. Pharmacological treatment with RAAS-inhibitors is the current golden standard in many of these conditions[51, 52]. Consequently, in addition to being a key component in the protective arm of the RAAS, sACE2 is also considered a biomarker of cardiovascular and cardiometabolic diseases when found elevated in the circulation.

Since the beginning of the pandemic and the early finding that SARS-CoV-2 uses the ACE2 as a receptor for cell entry and subsequent viral replication, ACE2 and the RAAS have attracted great attention[16, 53]. A RAAS-imbalance with an acceleration of the classical RAAS has frequently been proposed as part of the pathophysiology in COVID-19[54-56].

SARS-CoV-2 has a high affinity for both two forms of ACE2 [57]. However, the fate of the enzyme after SARS-CoV-2 interaction may differ between mACE2 and sACE2. In the case of mACE2, in the process of SARS-CoV-2 cell entry a simultaneously degradation of mACE2 will occur[57]. The loss of mACE2 will not only reduce enzymatic capacity at the cell surface but also decrease the amount of buffering sACE in the circulation, generating an increased ratio of ANG II/ANG 1-7. The same has not been described for SARS-CoV-2 interaction with sACE2, since sACE2 lacks an adherent cell membrane. Instead, the sACE2 remains catalytically active even though bound to circulating SARS-CoV-21 [42]. However, the fate of sACE already bound to SARS-CoV-2 when cell fusion occurs is currently

unclear[58]. Though it has been proposed that any sACE2 bound to SARS-CoV-2 will be lost in a fusion process[59].

Today, risk factors associated with severe COVID-19 are well described, most of which are factors previously known to be associated with increased levels of sACE2, i.e., diseases comprising the metabolic syndrome as well as male sex, and increased age[10, 60, 61]. The association between a pre-existing chronically activated classical RAAS (reflected by the levels of circulating sACE2) and the risk of developing severe COVID-19 when infected by SARS-CoV-2, suggest that viral interference with ACE2 may be an important link in the pathophysiology behind severe COVID-19.

2.5 THE METABOLIC SYNDROME

The metabolic syndrome is the term used for a combination of metabolic disorders strongly associated with cardiovascular morbidity and mortality[62]. The lack of any universally accepted definition of the metabolic syndrome has complicated comparison of prevalence estimates. Today, the most widely used clinical criteria for diagnosing the metabolic syndrome are those proposed by the WHO[63] and/or by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII)[64]. The conditions now widely accepted to be components of the metabolic syndrome include *hypertension, obesity, insulin-resistant glucose metabolism,* and *dyslipidemia*. Of note is the reports of an alarming increase in the prevalence of these conditions worldwide, making the metabolic syndrome another ongoing global pandemic, unfortunately not limited only to adults [65, 66].

Metabolic homeostasis is complex and involves many hormonal systems, each with their own axis and feedback system, in a finely tuned interplay. Unfortunately, we lack a biochemical marker for early recognition of imbalance in these systems. However, in dysglycemic metabolic disorders, diabetes mellitus and prediabetes, the glycated hemoglobin (HbA1c) test is used as a reliable marker for long-term glycemic status[67]. Hemoglobin in erythrocytes becomes irreversible coated (glycated) with glucose from the bloodstream and glucose levels will therefore be reflected on the surface of the hemoglobin. The test displays an average of the blood glucose level over the past three months, which is the normal life span of erythrocytes[67]. The HbA1c test has been proposed to be a useful marker for early detection of insulin resistance, a major etiological factor for the development of the metabolic syndrome, and to be included in the definitions of the metabolic syndrome[68-71].

All diseases constituting the metabolic syndrome are now known to be risk factors for severe COVID-19[72-76]. Even though the pathogenesis of the metabolic syndrome is not yet fully understood, a link between all components of the syndrome and a chronically unbalanced RAAS has been proposed for many years. The findings of elevated levels of circulating AEC2 in all the diseases of the metabolic syndrome strengthens this proposal[51, 52]. In addition, RAAS-blocking agents (i.e., ACE-inhibitor or angiotensin receptor blocker (ARB)) have become standard of treatment for several of the diseases in the metabolic syndrome with

improved status in all parts of the syndrome [35, 77, 78]. This is a further implication of a RAAS-involvement in pathogenesis of the metabolic syndrome.

2.6 THE RAAS AND ARGININE VASOPRESSIN

The principal anti diuretic hormone in humans is arginine vasopressin (AVP). This hormone is synthesized in the hypothalamus from a precursor protein (pre-provasopressin). Arginine vasopressin is then stored in vesicles within the posterior lobe of the pituitary and released into the circulation in response to appropriate stimuli. In the cleavage process from the precursor protein, an equimolar amount of the biologically inactive copeptin is produced. Copeptin is stored, transported, and secreted in equal ratio with AVP and is commonly used as a surrogate marker for AVP due to its pre-analytical stability and longer i*n-vivo* half-life[79-81]. The main stimulus for AVP release (and thus copeptin) includes high plasma osmolality, low circulating blood volume, hypotension, and ANG II. Acting on its receptors, the main effects of AVP are water reabsorption, vasoconstriction, and promotion of coagulation[82].

There is a close, and well-coordinated, interaction between the RAAS and the AVP system, and their joint action is necessary for the appropriate regulation of water-electrolyte balance, metabolism, and the cardiovascular system. Consequently, co-activation of the two systems in response to the same stimulus is common. In addition, ANG II is a potent stimulator of AVP release[83]. The electrolyte most associated with AVP is sodium. Being the main determinant of plasma osmolality, any change in plasma sodium concentration will ultimately influence AVP release.

Factors known to be associated with an immediate increase in AVP levels are viral pneumonias, hypoxia, inflammation, sepsis, and increased ANG II levels, whereas chronically elevation of AVP has been associated with the diseases comprised by the metabolic syndrome[84-88].

2.7 COVID-19 IN THE CLINICAL SETTING

When humans are infected with SARS-CoV-2, the clinical presentation varies from asymptomatic to severe infection. Most cases of *symptomatc* infection, or COVID-19, are mild to moderate without need for hospitalisation or advanced medical care (~80%)[9]. In the cohort of patients hospitalised for severe manifestations of COVID-19 in need of intensive care and mechanical respiratory support due to hypoxic repspiratory failure, approximately 5% detoriate and develop septic shock and/or multi organ failure[9].

The risk factors associated with severe COVID-19 are well described and includes advanced age, male sex, and aformentioned constituents of the metabolic syndrome[8, 89]. The occurence of one or more comorbidities in hospitalised COVID-19 patients has been reported to be as high as 60-90%[8, 90]. Even though people of all ages appears to be susceptible to SARS-CoV-2 infefection, most young people and children have a mild clinical course or are asymptomatic[91].

The most common clinical manifestations observed in a patient with severe COVID-19 include a history of 7-10 days of fever, dry cough, headache and a progressive shortness of breath[9]. Most patients hospitalised or admitted to ICU, are male (60-70%) aged 45-70 with an increased abdominal circumference. From our experience, the patients are ususally in a compliant and mentally lucid state despite a situation of severe respiratory distress with significant hypoxia and takypnea. Respiratory rate most often exceeds >20 breath/min, generating a respiratory minute ventilation of between 15-20 liters due to large tidal volumes. A common finding on admisson is an arterial blood gas showing severe hypoxia, normocapnia and normal lactate levels. Most patients exhibit preserved blood pressure on admisson, with no or low-moderate need of additional vasopressors[92-94].

The radiological findings include bilateral, peripheral ground-glass opacities and or consolidation[9].

The laboratory abnormalities most commnly encountered in hospitalised patients are elevated inflammation markers (eg. C-reactive protein, erythrocyte sedimentation rate, and ferritin), lymphopenia, abnormal coagulation parameters (eg. high fibrinogen, elevated D-dimer, low platlet counts), electrolyte disturbances (eg. hyponatremia and hypoalbuminemias)[95-97], and signs of liver involvement (eg elevation in levels of lactate dehydrogenase, triglycerides, aspartate aminotransferase, and alanine aminotransferase levels)[97]. In addition, elevated levels of glycated hemoglobin (HbA1c) are a frequent finding in hospitalised COVID-19 patients with or without a history of diabetes mellitus[98, 99].

The numbers of patients treated in the ICU in need of invasive mechanical ventilation varied over the course of the pandemic due to different ventilatory approaches. However, in mechanically ventilated patients, a common finding is preserved lung compliance despite a severe hypoxia and a high respiratory drive, displaying a P_aO_2/FiO_2 ratio fulfilling criteria for severe ARDS[100]. As a consequence, ARDS guidelines, recommending the use of controlled protective ventilation, were followed resulting in a persistent hypercapnic situation in many of the patients [101].

The findings of frequent tromboembolic events in COVID-19 patients[102], in combination with laboratory findings of abnormal coagulation and the clinical picture of significant impaired ventilation, resulted in the implentation of new anticoagulation treatment regimes for COVID-19 patients[103]. In addion to the complicating tromboembolic events observed, systemic and pulmonary hypertension, right ventricular failure, acute kidney injury and neurological impairment frequently complicates the course in severe COVID-19[104-107].

The clinical features of severe COVID-19 differs in many aspect from those previously seen in critical ill patients with severe pneumonia and ARDS[93]. In contrast to COVID-19, these patients are more often affected by sepsis[101]. Consequently, hypotension with hypoperfusion of vital organs are frequently observed and are associated with impaired counciousness and many patients require early inotropic and vasopressor support for the prevention of multi-organ failure. This contrast with the hemodynamic profile seen in severe

COVID-19 patiens where a hypertensive clinical course requiring anti-hypertensive treatment beyond sedation is frequently described[93, 94]. In non-COVID-19 associated ARDS, the lung mechanics usually differ as well, with a higher frequence of low lung compliance hypoxia on presentation compared to patients with severe COVID-19[100, 108, 109]. There are however, some smaller studies showing a time-dependent association between a high versus low lung compliance in COVID-19 patients[110].

The early proposal of a so-called cytokine storm in patients with COVID-19 has later been questioned and debated. When compared to conditions with commonly occuring cytokine storms, (i.e., sepsis, ARDS or T cell-induced cytokine release syndrome) the inflammtory cytokine elevations in severe COVID-19 has been shown to be profoundly lower[111]. In contrast, similar or greater elevations of several acute-phase reactants or non-cytokine biomarkers, including D-dimer, ferritin and C-reactive protein, have been found in patients with severe COVID-19 when compared to these disorders. However, this may not be generalisable to all, a subgroup of patients with severe COVID-19 does meet the criteria of cytokine storm[111, 112].

2.8 LACK OF KNOWLEDGE AND RATIONALE FOR THE THESIS

The early discovery that SARS-CoV-2 uses the ACE2 as a receptor for cell entry and subsequent replication was for many of us a moment of epiphany. The importance of the RAAS and its complexity was highlighted and a deepened understanding of the alternative RAAS was elucidated, i.e., the RAAS grew a new arm!

Being a vital but complex feed-back-feed forward system, the RAAS is engaged in far more physiological processes and responses than most often described (Table 1). For normal physiology, a strict balance between the two effector peptides of the two arms of the RAAS is crucial. Key to such balance is ACE2, the enzyme responsible for the conversion of abundant ANG II to the more protective peptide ANG 1-7 in the alternative RAAS. In situations of insufficient ACE2 activity of which SARS-CoV-2 infection is an example, a disruption in the balance between the two arms of RAAS may occur. This skews the system in favor of classical RAAS with increased ANG II effects (Figure 3).

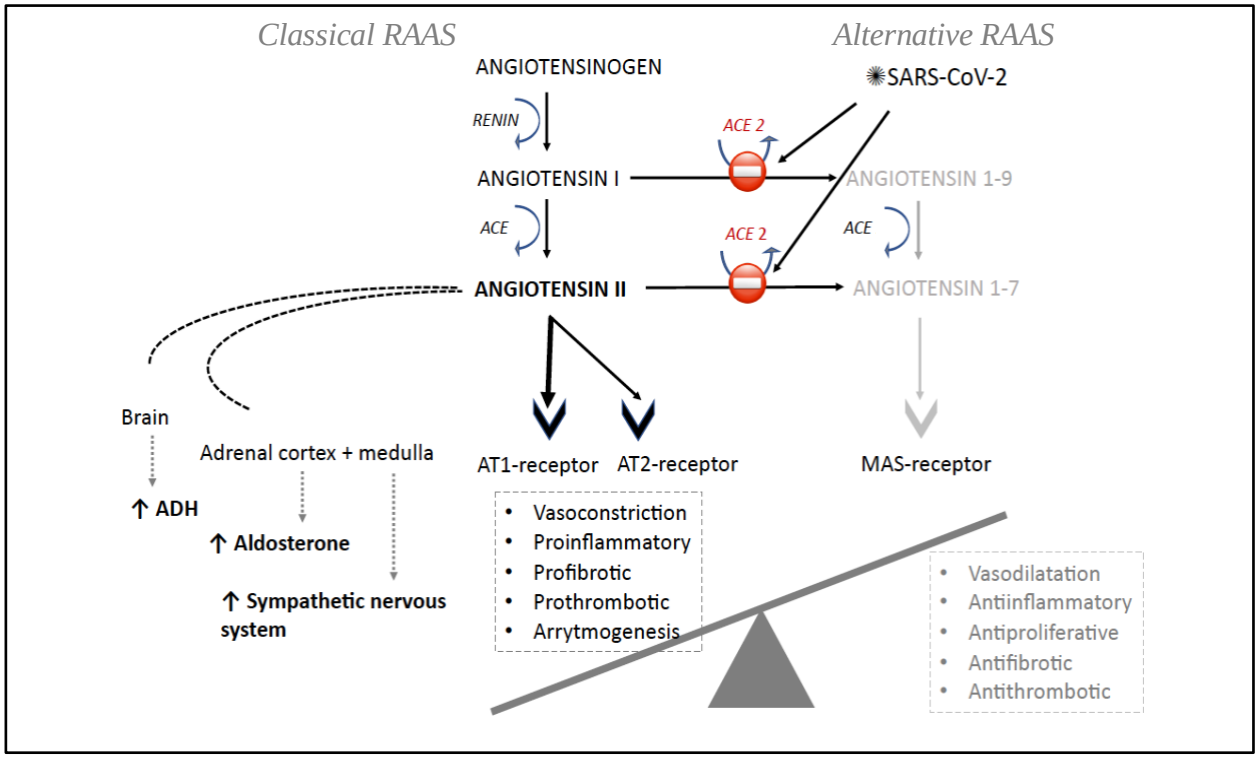

Figure 3. *A schematic illustration of an imbalanced RAAS, in this case caused by SARS-CoV-2 interference with ACE2 thus skewing the system in favor of classical RAAS. ACE= Angiotensin Converting Enzyme; ACE 2= Angiotensin Converting Enzyme type 2; ADH= antidiuretic hormone; AT1= Angiotensin II receptor type I; AT2= Angiotensin II receptor type 2.*

Interestingly, the many documented clinical effects following ANG II-AT1R interaction (Table 1) are the same as those encountered in many patients with severe COVID-19, including hypertension, elevated systemic and pulmonary artery pressure, thromboembolism, insulin resistance, inflammation etc., making a viral induced skewness of the RAAS likely. However, evidence supporting such RAAS imbalance in COVID-19 patients is limited. To further explore this, studies I, III and IV were designed.

Moreover, the early clinical observations that patients suffering from severe COVID-19 constituted a rather homogenic population, sharing many clinical features with those outlined for the metabolic syndrome, were soon confirmed in several publications. The characteristics most associated with a severe course of COVID-19, include advanced age, male sex and comorbidities comprising the metabolic syndrome[10, 73, 113, 114]. These are all conditions associated with increased levels of sACE2. This is indicative of a chronic activation of classical RAAS, since sACE2 represents shed catalytic domains from mACE2s in response to e.g., ANG II. Advanced age might be an exception, where loss of mACE2 may just be part of the aging process. These patients may not necessarily exhibit high levels of sACE2 but overall low ACE2 activity[115]. Hypothetically, when people with a premorbid skewness in the RAAS with excessive classic RAAS-activation contract SARS-CoV-2, viral binding to

mACE will generate an acute on chronic situation where further loss of ACE2 will further increase the RAAS-imbalance.

The link between the RAAS and metabolic syndrome has been known for some time and RAAS-inhibitors are now first-line treatment options for many of the included diseases[116]. To better understand the link between chronic metabolic dyshomeostasis and the risk of developing severe COVID-19, study II was performed using HbA1c as a marker of metabolic imbalance.

3 RESEARCH AIMS

The overall aim of this thesis was to study the pathophysiological pathways and the clinical presentation among critically ill COVID-19 patients with focus on viral interference with the renin-angiotensin-aldosterone system.

The specific aims were:

- To investigate if a disturbance in the RAAS similar to the one proposed for SARS-CoV-2 contributes to the pathophysiological syndrome of COVID-19.

- To assess the association betweeen chronic dysglycemia, diagnosed by analysis of available HbA1c, and SARS-CoV-2 associated respiratory failure in hospitalised patients with a positive PCR-test for SARS-CoV-2.

- To examine the dynamics of routine plasma electrolytes and acid-base biomarkers in patients with severe COVID-19 and if they were associated with the need for mechanical ventilation and/ or mortality.

- To compare the circulating concentrations of three main biomarkers of the RAAS (ANG II, ANG 1-7 and ACE2) and arginine vasoopressin (copeptin) in a cohort of COVID-19 patients admitted to the intensive care unit compared to healthy controls.

4 MATERIALS AND METHODS

4.1 ETHICAL CONSIDERATIONS

The Swedish Ethical Review Authority, approved studies I-IV, which were performed in accordance with the ethical standards laid down in the Declaration of Helsinki. The experimental part of Study I was approved by The Swedish Board of Agriculture and carried out in accordance with The Swedish Animal Welfare Agency.

In all studies, thoroughly consideration of ethical issues was made. It was concluded that the benefits exceeded any potential harm. The SARS-CoV-2 pandemic was unique, in that a large number of patients suffering from a new and rapidly spreading viral disease, in need of advanced medical support simultaneously, challenged healthcare systems all over the world. The need for a better understanding of the disease was imperative for preventive measures, risk assessment and for developing treatment options. Performing a large animal experiment during an ongoing pandemic was complicated but generated valuable information regarding the association between a disruption of the RAAS and the development of disease as well as possible treatment options.

Early in the pandemic, local, regional, and national COVID-19-databases were established. These databases have become important tools to acquire a better understanding of the disease COVID-19.

All patient data from the four studies were pseudonyminised according to ethical regulations in Sweden and are kept in in locked storage at Clinical Research Units at the Karolinska University Hospital, Stockholm, Sweden.

All studies were performed during the pandemic.

4.2 STUDY DESIGN AND OUTCOMES

Table 2. *Study design and outcomes for the included studies.*

	Study I	Study II	Study III	Study IV
Design	Translational study	Retrospective observational cohort study	Retrospective observational cohort study	Prospective observational study
Study period	April-October 2020	March-September 2020	March-April 2020	November 2020-May 2021
Total number of subjects included	16 swines 289 + 2 patients	385 patients	428 patients	56 patients 10 healthy controls
Interventions	Experimental pharmacological interference with the RAAS, MRI, Echocardiography, blood samplings.	None	None	Blood sampling
Outcome	Clinical correlation of CTPA, MRI-perfusion and pulmonary artery pressure with a swine model of RAAS-imbalance.	The development of SARS CoV-2 - associated respiratory failure.	Requirement for mechanical ventilation and mortality.	Description of the levels of ANG II, ANG 1-7, ACE2 and copeptin in severely ill COVID-19 patients in relation to a healthy control group
Analyses	Two-way ANOVA with Dunn-Sidak correction. Mixed error-component models.	Chi-square test. Mann-Whitney U-test. Logistic regression.	Shapiro-Wilk test. Chi-square teta. Logistic regression Kruskal-Wallis test Mann-Whitney U-test. Wilcoxon signed ranks test	Chi-square test. Mann-Whitney U-test. Logistic regression

4.3 PARTICIPANTS

Patients included in the studies within this thesis were adults (>18years) with positive SARS-CoV-2 PCR test admitted to Karolinska University Hospital, Sweden during the Swedish first, second or third wave. None of the patients had been vaccinated against SARS-CoV-2 prior to inclusion.

Information regarding previous medical history, pharmacological treatment and routine laboratory tests was obtained by review of the patient´s medical records (Take Care) and from available electronic patient data management systems (Centricity Critical Care).

In study I, the patients included in the retrospective imaging cohort were identified in the Picture Archiving and Communication System (PACS) at Karolinska University Hospital. The two ICU-patients included were identified by the treating physician.

Patients in study II were identified in the Karolinska University Hospital COVID-19 quality database. This database was established during the early part of the pandemic with the aim of improving the quality of care for patients with SARS-CoV-2.

In study III, all included patients were identified in the Karolinska University Hospital laboratory database, Database Flexlab.

The patients included in study IV were identified on ICU-admission, primarily by research nurses. Ten healthy volunteers were also included, all without any history of COVID-19 or COVID-19-vaccination. All controls were volunteers who were identified in the hospital environment and included if inclusion criteria were meet.

4.4 LARGE ANIMAL EXPERIMENT, STUDY I

The pathophysiological consequences of a RAAS-imbalance due to insufficient ACE2 activity, were investigated in a series of experiments. The studies took place at the Karolinska Experimental Research and Imaging Center (KERIC), Karolinska University Hospital, Sweden.

All animal procedures were performed according to the KERIC guidelines for animal research at Karolinska Institutet, Sweden.

After preoperative handling including, anesthesia, intubation and ventilation, the animals were placed in a supine position for ease of access to all catheters. We used continuous routine monitoring for general anesthesia (i.e., a five lead ECG, arterial pulse oximetry, and end-tidal CO_2 monitoring). To monitor continuous central blood pressure and for arterial blood gas analysis, a femoral arterial line was inserted and placed in the aorta arch. In addition, a central venous catheter was placed in the internal jugular vein for administration of drugs. For measuring pulmonary vascular status during the experiment, a trans-femoral pulmonary artery catheter was inserted. All animals received a urine catheter and hourly diuresis was recorded, (Figure 4.).

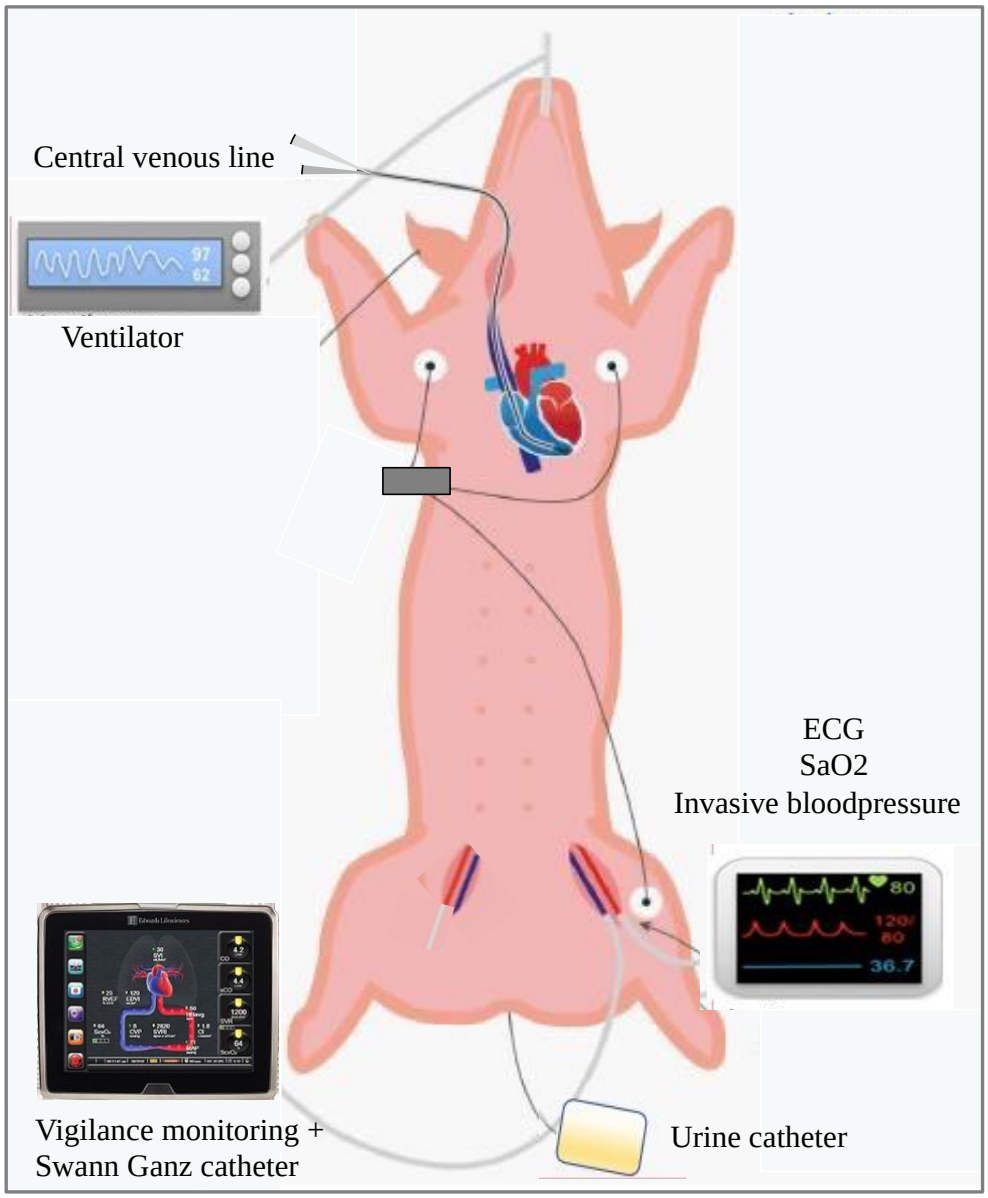

Figure 4. *Experimental set up for study I. ECG= electrocardiography; SaO_2=arterial oxygen saturation.*

The experiment comprised five different protocols, including three different approaches to achieve pharmacological RAAS-imbalance, one in which a treatment regimen was evaluated, and finally one scenario with placebo treated animals without induced RAAS-imbalance (Fig 5).

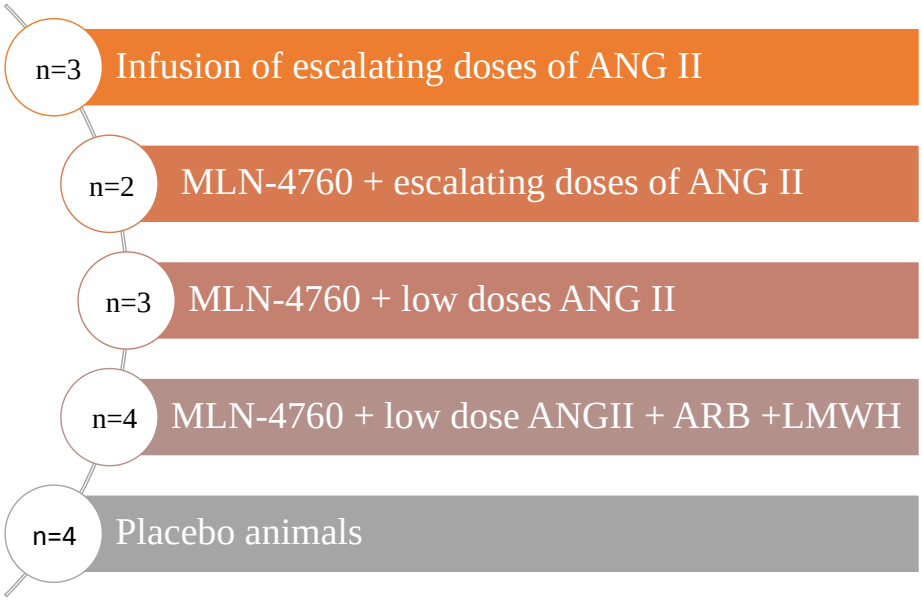

Figure 5. *Study I. Illustration of the large animal experiment and the constituent groups. ANG II=angiotensin II; MLN-4760= a selective ACE2-inhibitor; ARB= angiotensin receptor blocker (Losartan®); LMWH= Low-Molecular-Weight Heparin.*

The drugs used to induce a RAAS-imbalance were ANG II and MLN-4760, a selective ACE2-inhibitor. In all groups, scheduled pre-clinical chemistry, hourly diuresis measurement, pulmonary angiography, physiological data collection, screening for DVT, bleeding-time assessment and post-mortem analysis were performed. In addition, Magnetic Resonance Imaging (MRI) with lung perfusion was performed in swine 5-16. Echocardiography was performed in two animals, one in the treatment group and one with RAAS-imbalance for comparison. MRI was performed on a Siemens MAGNETOM Aera 1.5 Tesla scanner. An anatomical 2D motion-corrected T_2-weighted sequence was used to identify pulmonary infiltrates. For the dynamic contrast (Gadolinium) series, a 4D time-resolved MRI angiography was used. Pre-clinical histological analyses performed after the preparations were initially placed in 4% formaldehyde for 24 hours and then the formaldehyde was exchanged. The samples were cut and stained using Mayer`s Hematoxylin and Eosin prior to scanning and analysis.

4.5 BLOOD SAMPLINGS AND ANALYSES

In Study I, pre-clinical chemistry analysis was performed at the Karolinska University Laboratory, Sweden. Blood samplings were performed at baseline and at 120, 240, 360 and 435 minutes. These included test for coagulation, hematology, infection and common cytokines, electrolytes, kidney function, and liver function. Arterial blood gases were obtained at baseline and at 75, 270, 345, 435, 545, 665, 750 minutes.

In Study IV, five milliliters of blood was collected in EDTA plasma preparation tubes with filter within 24 hours from ICU admission. The samples were then placed on ice and centrifugated at 1200 g for 10 minutes at 4ºC within 30 minutes of sampling. The samples

were stored at -80 ºC. An Enzyme-Linked Immunosorbent Assay (ELISA) kit was used for detection of ACE2, ANG II, ANG 1-7 and Copeptin in plasma. Samples were analysed in duplicates and the mean of the two values was finally used.

4.6 STATISTICS

For all studies

Continuous data are presented as mean and standard deviation or as median and interquartile range as appropriate. Nominal data are presented as frequencies and percentages. Statistical significance was set at 0.05.

Study I

In the preclinical part of the study comparison between groups was performed by ANOVA with Dunn-Sidak correction for multiple comparisons since there was an uneven number of individuals in the groups.

Longitudinal physiological data was analysed using a mixed error component model with restricted maximum likelihood and an unstructured covariance matrix. Equations were designed with the response variable of a physiological parameter with fixed effects investigated for group status and random intercepts and slopes for each individual. The group membership variable was also allowed with linear time to capture differences in growth rates between the groups. Further, a variable capturing unmeasured time-varying characteristics on the individual level was also included in all equations. Final p-values were corrected for multiple comparisons using the Sidak-Holm adjustment. For the retrospective imaging cohort, the intraclass correlation coefficient was calculated between the two readers.

Study II

In this study, categorical data were compared using the chi-square test and the Mann-Whitney U test were used for continuous data. For the primary aim, the association between chronic glucose control (no diabetes, prediabetes, unknown diabetes, controlled diabetes, and uncontrolled diabetes) and HbA1c strata was assessed using multivariable logistic regression analyses and adjusted for age, sex, and BMI. In a sensitivity analysis, adjustments for baseline variables with a p-value <0.1 on univariable analysis was performed. The Hosmer-Lemeshow test and area under the receiver operating curve (ROC) were used to determine goodness of fit and model discrimination.

Study III

In this study, logistic regression was used to investigate the effect of clinical variables on primary aim, i.e., the risk of death and the risk of requiring mechanical ventilation. For the assessment of any group differences, the Kruskal-Wallis test was used and Mann-Whitney U-test was used for pairwise comparisons. To evaluate any change in plasma hematocrit between admission and day nine (i.e., day of peak sodium within the groups) Wilcoxon rank

sum test was used. Normality of data was assessed using Shapiro-Wilk test and a two-sided Chi-square test was used to evaluate any differences in distributions between the groups.

Study IV

Categorical data are presented as numbers and percentages and compared using the chi-squared test. Continuous data are presented as median with interquartile range and compared using the Mann-Whitney U-test.

5 RESULTS

5.1 STUDY I

In this study we explored the pathophysiological consequence of a pharmacologically induced RAAS-imbalance by activating the classical RAAS by infusion of ANG II, with or without simultaneous administration of an ACE2-inhibitor. In addition, data from a cohort of SARS-CoV-2 positive patients examined with computed tomography pulmonary angiography and two critically ill COVID-19 ICU-patients were used for comparison.

In our large animal experiment where an acceleration of classical RAAS was induced, we found a pathophysiological state, including reduced lung perfusion, elevated pulmonary artery pressure, reduced PaO_2, diffuse alveolar damage, coagulation abnormalities, and signs of acute tubular necrosis, that shares several features seen in severe COVID-19 patients. The state could be ameliorated with RAAS-inhibitors and Low-Molecular-Weight Heparin (LMWH). On lung perfusion MRI, a marked reduction in blood flow was observed in animals with induced but untreated RAAS-imbalance (Figure 6).

In the retrospective cohort of COVID-19 patients we found multimodal evidence of elevated pulmonary artery pressure (i.e., CTPA and echocardiography) and frequent pulmonary thromboembolism (17%). The elevated pulmonary artery pressure is in line with the invasive pulmonary artery pressure observed in a critically ill COVID-19 patient with a Swann-Ganz catheter in situ (day 25), showing pulmonary hypertension. In another critically ill COVID-19 patient, MRI lung perfusion was performed (day 22), showing significant perfusion disturbances, mainly in the peripheral parts of the lungs (Fig 6 d). We found no clear evidence indicating a cytokine release syndrome in either of the cohorts.

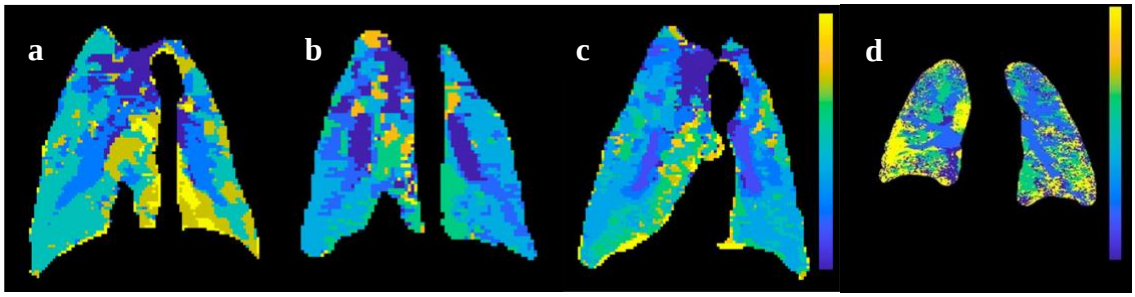

Figure 6. *MRI perfusion in swine (a-c) and in a patient with severe COVID-19 (d). Time-to-peak parametric map with the color look up table set to maximum blue for pulmonary artery peak and yellow for aortic peak or later, e.g., yellow indicate late arrival compatible with disturbed microcirculation.* **a** *Swine lung after administration of low dose ANG II and ACE2 inhibitor MLN-4760.* **b** *Placebo swine.* **c** *Swine lung after administration of low dose ANG II and ACE2 inhibitor MLN-4760 and the combination of oral angiotensin receptor blocker and low molecular weight heparin.* **d** *Lungs from a patient with severe COVID-19 (day 22). Note: human lungs not scaled.*

5.2 STUDY II

Study II was a retrospective observational study where we assessed the association between chronic dysglycemia, determined by analysis of HbA1c, and SARS-CoV-2 associated respiratory failure in hospitalized patients with confirmed SARS-CoV-2 infection. Of the 385 patients included in the study, 78% suffered from respiratory failure. Median BMI and median HbA1c was higher in patients with respiratory failure. In patients in need of intensive care due to respiratory failure, chronic dyslycemia was observed in 83% (Fig.7).

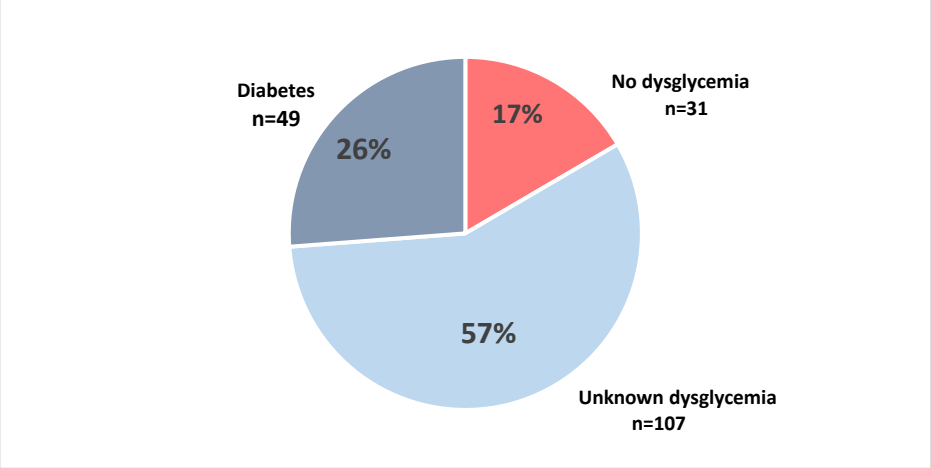

Figure 7. *The proportion of patients with dysglycemia admitted to ICU due to SARS-CoV-2 associated respiratory failure, n=187. ICU= intensive care unit*

Further, almost half of the patients developing respiratory failure were found to have an undiagnosed chronic dysglycemia and 26% had a poorly controlled diabetes. Among patients without respiratory failure, these numbers were significantly lower, 13% and 7% respectively (Figure 8).

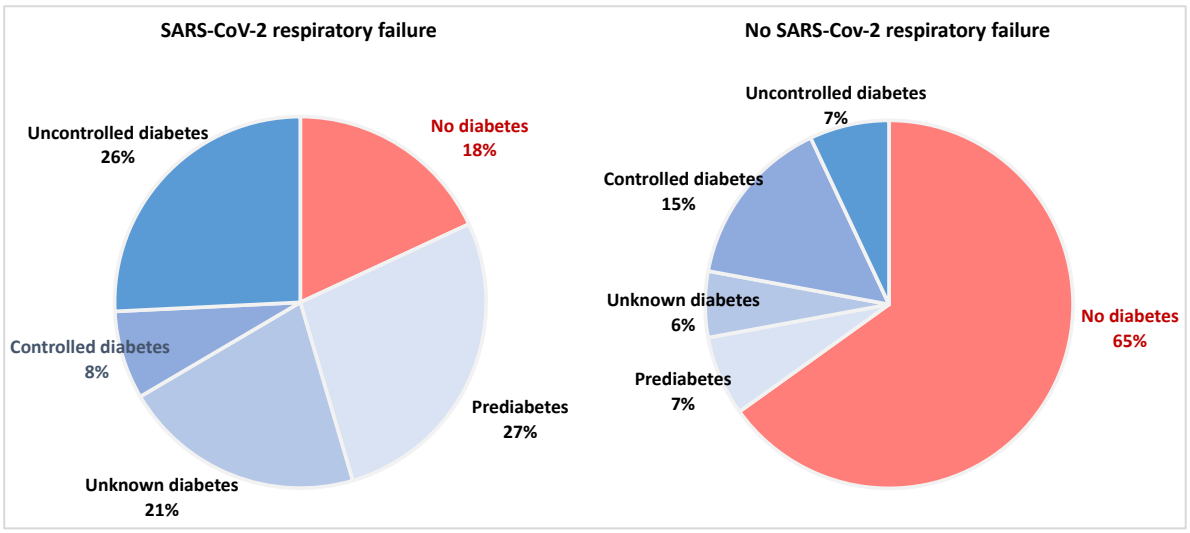

Figure 8. *The proportions of patients with dysglycemia in hospitalized SARS CoV-2 patients developing respiratory failure or not.*

In multivariable logistic regression analysis, prediabetes, unknown diabetes, and uncontrolled diabetes were all, independent of age, sex, and BMI, associated with respiratory failure (Table 3).

Table 3. *Univariable and multivariable logistic regression analysis showing the association with SARS-CoV-2 induced respiratory failure. BMI= body mass index; CI= confidence interval.*

	Univariable		Multivariable[a]	
Variable	Odds Ratio (95% CI)	*p*-value	Odds Ratio (95% CI)	*p*-value
Male sex	1.52 (0.90–2.57)	.116	1.91 (0.97–3.80)	.06
Age, years	1.01 (0.99–1.02)	.542	0.99 (0.97–1.01)	.24
BMI, kg/m^2	1.09 (1.03–1.16)	.005	1.06 (0.99–1.13)	.09
Chronic dysglycemia				
No chronic dysglycemia	1.00			
Prediabetes	14.17 (5.71–35.19)	<.001	14.41 (5.27–39.43)	<.001
Unknown diabetes	13.07 (4.88–34.97)	<.001	15.86 (4.55–55.36)	<.001
Controlled diabetes	1.83 (0.84–3.99)	.125	2.08 (0.84–5.15)	.11
Uncontrolled diabetes	13.31 (5.35–33.10)	<.001	17.61 (5.77–53.74)	<.001

5.3 STUDY III

Study III was a retrospective observational study where we examined the association between electrolyte and acid-base dynamics and the requirement for mechanical ventilation and mortality (30-day) in hospitalised patients with severe COVID-19. This study was performed during the first wave of the pandemic. In total, 406 patients were included. Hyponatremia on admission was observed in 57% of the patients and were found to be associated with requirement for mechanical ventilation. Over time, 42% developed hypernatremia within two weeks and with a median sodium peak on day nine. Hypokalemia was a frequent observation in patients with hypernatremia (Fig. 9). The development of hypernatremia was associated with a more severe course of the disease (i.e., ICU admission, mechanical ventilation, and renal replacement therapy) and a higher risk of death compared to patients without hypernatremia (OR 3.9 (95% CI 2.27-6.85)).

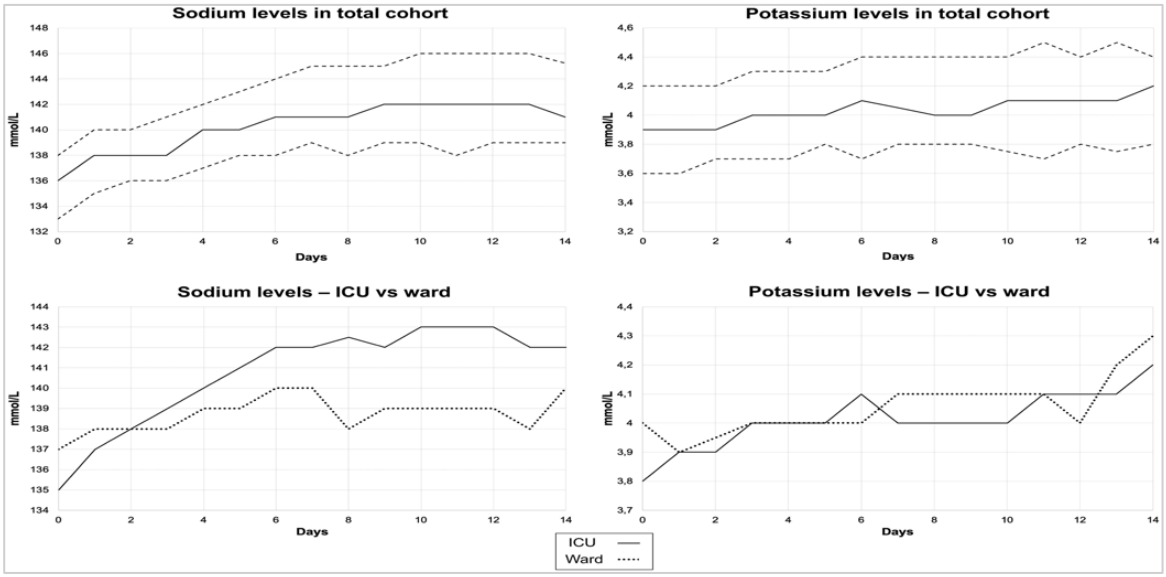

Figure 9. *Sodium and potassium dynamics over time in all patients (n=406), during the first two weeks of hospitalization. Upper row is median sodium and potassium levels (solid lined) with upper and lower quartiles (broken line. Bottom row is median sodium and potassium levels of patients with (n=299) or without (n=107) the need for intensive care.*

5.4 STUDY IV

In study IV, the aim was to measure and compare circulating levels of sACE2, ANG II, ANG 1-7, and Copeptin (arginine vasopressin, AVP) in COVID-19 patients admitted to ICU and in ten healthy controls.

In all, 56 patients were included of which 70% were men. On ICU-admission, blood pressure was preserved, and hypotension or shock states were uncommon. On ICU routine laboratory testing, elevated HbA1c (median of 54 mmol/mol (IQR 47-68)), hyponatremia (54%) and low plasma albumin (median 24 g/L (IQR 22-27) was observed. The plasma levels of sACE2 and copeptin were lower in COVID-19 patients compared to healthy controls but without any difference in the levels of ANG II or ANG 1-7 between the groups (Fig 10).

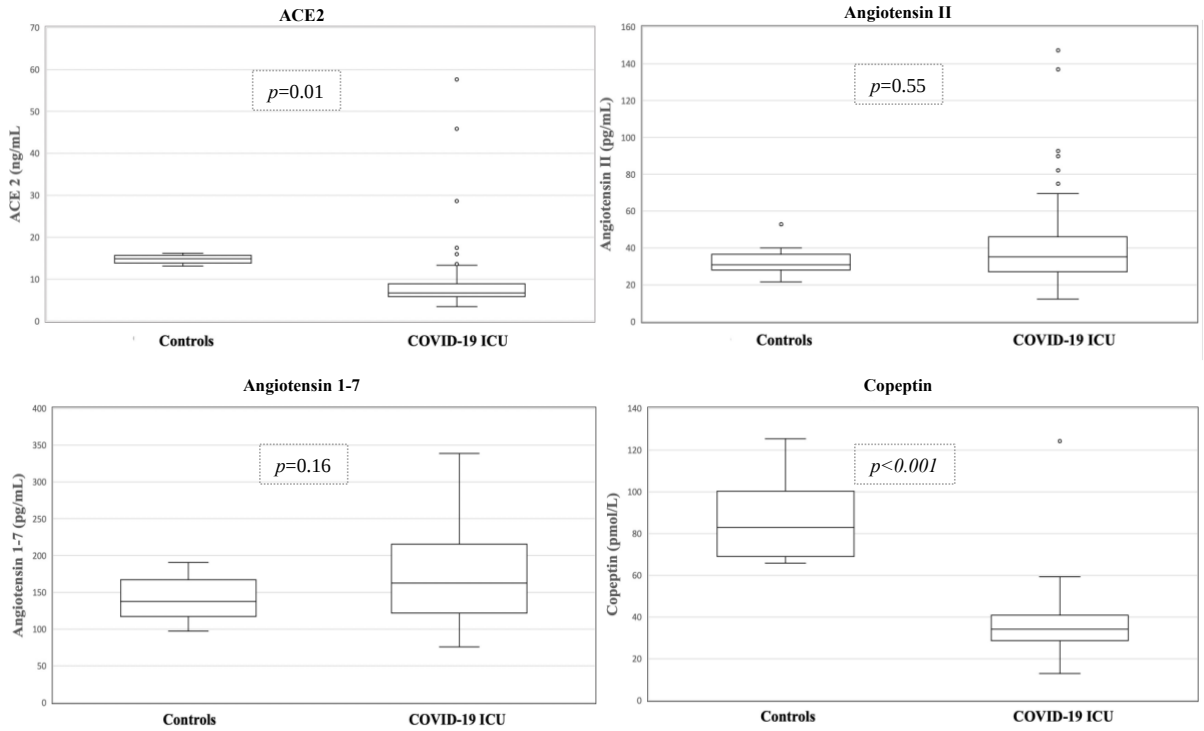

Figure 10. *Plasma concentrations of three biomarkers of the RAAS (sACE2, ANG II, ANG 1-7) and Copeptin, in COVID-19 patients admitted to the ICU (n=56) and in healthy controls (n=10). RAAS: renin-angiotensin- aldosterone system; ICU= intensive care unit; ACE2 angiotensin converting enzyme type 2; ANG II= angiotensin II; ANG 1-7= angiotensin 1-7.*

6 DISCUSSION

This thesis focused on pathophysiology in severe COVID-19 and the association with the RAAS and the metabolic syndrome. We found that in a translational fashion, pharmacological acceleration of the classical RAAS generates a pathophysiological syndrome that shares several features of COVID-19, including reduced lung perfusion and alveolar damage, elevated pulmonary artery pressure, reduced arterial oxygen saturation, coagulation disturbances, and acute kidney injury.

We also found that the presence of prediabetes, unknown or uncontrolled diabetes, diagnosed by available HbA1c, was associated with a markedly elevated risk of respiratory failure in a cohort of hospitalised SARS-CoV-2 positive patients.

Additionally, low levels of sodium on admission were associated with increased risk of mechanical ventilation whereas an in-hospital rapid developing hypernatremia was associated with increased length of stay and death. Moreover, lower pH on admission was associated with both death and need for mechanical ventilation.

Finally, in a prospective observational study, we found SARS-CoV-2 associated hormonal alterations in a cohort of critically ill COVID-19 patients, expressing low levels of circulating sACE2 and copeptin but preserved levels of ANG 1-7 and Ang II

This discussion will start with the viral interaction in human physiology and the likeliness of a viral induced skewness of the RAAS, i.e., study I and IV.

In study I, by pharmacological manipulation of the RAAS, with infusion of angiotensin II, alone or in combination with a ACE2-inhibitor (MLN-4760), the potency of ANG II became clear, and when uninhibited the actions of this hormone proved to be incompatible with life. However, in our more fitted model, when a more modest dose of ANG II was infused continuously over time in combination with MLN-4760, many of the clinical features of COVID-19 could be reproduced. Since patients with severe COVID-19 have a median time from infection to hospital admission of eight days[9], any potential RAAS-imbalance must evolve at a moderate pace, otherwise median time to presentation would be earlier. Consequently, this method of creating a slowly evolving RAAS-imbalance was chosen to mimic real COVID-19 as much as possible.

The most prominent feature of severe COVID-19 is a severe respiratory failure. The aetiology and clinical significance of the radiological findings of bilateral, peripheral ground-glass opacities has been debated. However, with the finding of frequent thromboembolic events in these patient, peripheral microvascular thrombosis has been proposed as one aetiology for many of the observed clinical features in COVID-19, including the respiratory failure. Unfortunately, the radiological tools to visualize microvascular thrombosis are limited and most of the evidence supporting this theory comes from autopsy reports.[117, 118]. To approach this problem, Magnet Resonance Imaging (MRI)with lung perfusion was performed in the study and in a few ICU-patients with COVID-19 (of which one was

included in the study). The marked reduction, and in some areas a total cessation, of blood flow found on these images in both animal and COVID-19 patients, confirmed a significant circulatory impairment in the lungs, even in areas without infiltrates (Figure 6). This indicates a vascular or a combined aetiology for the findings. Unfortunately, the aetiology of this impairment (i.e., thrombosis or vasoconstriction) could not be determined with this modality. Though, the elevated pulmonary artery pressure found both in the experimental and clinical setting suddenly became comprehensible.

At the time for the experiment, during the first wave of the pandemic in Sweden (i.e., March to June, 2020) treatment guidelines quickly changed. The findings of frequent thromboembolic events resulted in new guidelines recommending intensified treatment with anticoagulants being introduced. In our experimental model, one group were administered low molecular weight heparin (LMWH) in combination with an oral angiotensin receptor blocking agent (ARB), Losartan® in addition to the low dose ANG II-infusion regimen. The findings of improvements in several parameters in this group, including lower pulmonary artery pressure, improved lung perfusion and improved right ventricular function on echocardiography, were perhaps unsurprising but nevertheless, interesting, and in a future perspective promising. Early in the pandemic, the finding of a viral interference in the RAAS led to an intense debate regarding pros and cons concerning the continued use of RAAS-inhibitors for patients previously prescribed these preparations. Continued use is now recommended[119]. Moreover, there are several clinical studies, some of which are still ongoing, evaluating treatment with RAAS-blocking agents in COVID-19[120-124]. Unfortunately, these studies are not conclusive in whether RAAS-inhibitors are a treatment option in severe COVID-19 or not. This may in part be explained by the different designs and different populations included in these studies [120-122, 125]. However, as a potential treatment in the severely ill COVID-19 patient, today, there are no available intravenous preparations of ARBs on the market. This is unfortunate since the oral route is in many circumstances not the most suitable route for administration in ICU-patients.

The conclusion from Study I, that severe COVID-19, in part, may be driven by an overactivation of the classical RAAS with abundant ANG II effects, indicates that the point of interaction between SARS-CoV-2 and human physiology (i.e., the ACE2) may be an important link for outcome when infected with SARS-COV-2. Being a crucial component for balance within the RAAS, a patients ACE2 premorbid status may be associated with in COVID-19 severity.

To further investigate the RAAS and its involvement in severe COVID-19, study IV was performed. Levels of ANG II, sACE2, ANG 1-7, and AVP (copeptin) were analysed on ICU-admission and compared to a matched healthy control group. The rationale for including AVP in the analyses were the close interaction between the two hormonal systems (RAAS and AVP), the clinical observation of frequent hypertension in ICU COVID-19 patients, and the common finding of dysnatremia in this population. The latter being investigated in study III.

The findings in study IV were somewhat unexpected, with low levels of sACE2 and copeptin in combination with preserved levels of ANG II and ANG 1-7 as well as preserved blood pressure. The low levels of sACE2 observed are contrary to what would have been expected in this cohort of metabolically co-morbid patients, in whom increased levels of sACE are commonly reported[51, 52]. The low sACE2 levels observed could be the consequence of viral degradation of mACE2 with reduced ability to increase sACE2 by the shedding process. However, low levels of sACE2 would normally be accompanied by low levels of ANG 1-7, since the route of conversion of ANG II to ANG 1-7 by ACE2 is the primary route for ANG 1-7 formation[43, 49]. The findings of preserved levels of ANG 1-7 despite low sACE indicates an ongoing ANG II degradation. One possible explanation could be that when sACE is bound to a virus spike-trimer, it may become unanalysable but retain its catalytical activity. Considering the numerous viral spikes available for ACE2-binding, a significant amount of sACE2 may be attached to the surface of each virion. This could in theory have implications for our native immune response, not reacting rapidly enough to the infection due to the virions being well covered with the native human protein sACE2. This could be particularly problematic in a sACE2 rich environment (e.g., in patients expressing high levels of sACE2) where the virions may be highly covered with sACE2, due to the SARS-CoV-2 high affinity for ACE2. Further, if this virus-sACE2-complex eventually binds to a mACE2, the attached sACE2 may be lost in the cell fusion process, further aggravating the RAAS-imbalance.

Another unexpected finding that needs to be addressed in Study IV is the low levels of copeptin observed in combination with normal ANG 1-7 and ANG II and with preserved blood pressure. One would have anticipated with these concentrations of biomarkers (copeptin, ANG1-7 and ANG II) that patients would be hypotensive due to vasoplegia. This was not what was found in this study, where a median mean arterial blood pressure (MAP) on admission of 93 mmHg was observed. In addition, routine ICU blood samples revealed a high proportion of hyponatremia and hypoalbuminemia in the cohort, further complicating the interpretation of the finding of low copeptin concentrations. Most COVID-19 patients display a subacute onset of illness prior to hospital admission. During this time, fever, loss of appetite, gastrointestinal symptoms, etc. will activate the AVP-axis. Further, viral pneumonias, hypoxia, and inflammation are factors known to be associated with an immediate increase in the levels of AVP. Taking these circumstances in to account, the findings of hyponatremia and hypoalbuminemia in this study could be the result of a sudden AVP-depletion and consequently low copeptin levels. Since ANG II is a potent stimulator of AVP-release, any process of depletion could be further accelerated in a situation of classical RAAS-activation.

A similar AVP-depletion has been described in patients with septic shock, indicating a limited reservoir or storage pool of the hormone and an insufficient production in a high demand situation[126].

Finally in Study IV, preserved levels of ANG II were another surprising finding and are contrary to the hypothesis outlined prior to the study. In analogy with the proposed theory of a viral induced RAAS-imbalance as part of the pathophysiology in COVID-19, elevated levels of the hormone ANG II were expected. An explanation for the finding may be shortcomings or interference of biochemical analysis. Many of our hormones are short-lived compounds in vivo and are challenging to analyse. Being highly potent in their nature, a rapid clearance from circulation is essential for homeostasis. Clearance from circulation may be via a specified process (e.g., re-uptake, hydrolysis, conjugation etc.) but can also include receptor binding and in that way clearance from circulation. Therefore, surrogate compounds, formed in the processes of synthesis or degradation of these hormones, are often used for analysis [79, 127, 128]. In the case of ANG II, a defined surrogate compound does not exist. However, any of the degradation products, including ANG 1-7, or angiotensin III or IV could be used as such. (Angiotensin III and angiotensin IV are generated from ANG II through cleavage and display effects similar to ANG II but are less potent).

There may be several interpretations of the different findings in Study IV. However, the combination of normal to slightly elevated levels of ANG 1-7, despite low levels of ACE2, in combination with a blood pressure that might be considered high for an ICU-patient admitted for respiratory failure, implicates an ongoing ANG II degradation and the presence of a vasopressor other than AVP. Perhaps analyse of tissue ANG II in addition to plasma ANG II had further clarified these findings[25].

The consequence of SARS-CoV-2 interference with ACE2 and thus RAAS in patients with diseases associated with a pre-morbid chronic RAAS-imbalance with classical RAAS activation (i.e., hypertension, diabetes mellitus, obesity etc.) is somehow logical and could be described as an acute on chronic metabolic state. However, the reason why patients without any obvious risk factors or any previous medical history become severely ill when contracting SARS-CoV-2, whilst other do not, is not equally obvious.

The purpose of study II was to make this more comprehensible and explore the link between pre-morbid glycemic metabolic status (HbA1c) and COVID-19 severity in hospitalised patients with confirmed SARS-CoV-2. The main findings in this study, that unknown or uncontrolled chronic dysglycemia, were associated with respiratory failure in hospitalised SARS-CoV-2 positive patients was in accordance with our hypothesis. Similarly, normoglycemic SARS-CoV-2 positive patients, were predominantly hospitalised for non-COVID-19 diagnosis and SARS-CoV-2 was only an incidental finding. These results have also been confirmed by others[98, 129].

The large difference in proportions of patients with undiagnosed chronic dysglycemia and poorly controlled diabetes in the different groups (Figure 8.) highlights not only the high proportion of severely ill COVID-19 patients with a chronic metabolic dys-homeostasis, but also the value of a retrospective biomarker revealing pre-morbid health status, i.e., HbA1c. The findings that patients with unknown or poorly controlled chronic dysglycemia make up a significant proportion of those suffering from severe COVID-19 is an important finding that

makes HbA1c an valuable screening tool. However, this biomarker cannot detect all disturbances in metabolism. Some patients, despite normal levels of HbA1c, still develop respiratory failure due to SARS-CoV-2. Ideally, a test like HbA1c, assessing long- term status of other conditions, i.e., blood pressure or inflammation, would be of great value here. Such biomarker could have an impact for public health even beyond COVID-19.

Another interesting finding was the low number of patients with diabetes mellitus type 1 in this study (n=12), where only one, a patent on immunosuppressive therapy, developed respiratory failure due to SARS-CoV-2. The different pathophysiological processes underlying the two types of diabetes mellitus (metabolic versus immunologic) may be one reason for this finding.

Finally for study II, the proportions of patients with known hypertension did not differ between the groups nor was any difference in the proportions of patients on pharmacological anti-hypertensive treatment observed. If the proposed skewness of the RAAS is the pathogenesis in COVID-19, one would assume that patients on RAAS-blocking agents would be protected against a severe course of the disease. This was not seen in this study. However, in analogy with the findings of dysglycemia, where the risk of respiratory failure due to SARS-CoV-2 was markedly increased in patients with poorly controlled diabetes, this could apply to patients with insufficiently or poorly control hypertension as well. We have no biochemical method of detecting chronic poor compliance or poor hypertensive control. In addition, tools for identifying patients with chronic borderline hypertension or unknown hypertension are few. This group may well constitute a portion of the patients with respiratory failure but with normal range of HbA1c, i.e., their risk factor has remained undetected.

A strong association between severe COVID-19 and the diseases included in the metabolic syndrome is again noticeable in Study II. Increased BMI and a high frequency of diabetes and hypertension was observed among those suffering from severe COVID-19. Consequently, a person’s pre-morbid metabolic status seems crucial when infected with SARS-CoV-2.

The rational for study III was the clinical observation of frequent fluctuations in electrolytes in ICU-patients with COVID-19. The one most frequently observed was a moderate to severe hypernatremia. Even though dysnatremia is a common finding in ICU-patients, the combination of a high incidence in the COVID-ICU, a typical pattern of the evolving hypernatremia, and the close link between sodium and the RAAS, needed further exploration.

In this study we could demonstrate that both hypo- and hypernatremia was common in severe COVID-19, and both were associated with poorer outcome, i.e., need for mechanical ventilation or death. In addition, lower pH on admission was also a marker of worse outcome. However, this finding could reflect a more pronounced respiratory failure with elevated carbon dioxide affecting the acid-base balance rather than any electrolyte disturbance.

The high proportion of hyponatremia observed on admission in this study are well in line with the findings in study IV as well as with others[130]. However, this may not be the finding most anticipated in critically ill patients admitted after having been affected by an infectious illness for over a week. Instead, the most expected finding, in a patient with a history of prolonged fever, loss of appetite, cough, and gastrointestinal losses, would probably be increased sodium levels due to dehydration. Thus, a state associated with a prompt response of AVP release. In addition, viral pneumonias and hypoxic conditions, are associated with the syndrome of inappropriate secretion of antidiuretic hormone or AVP (SIADH) from the pituitary. This is a common cause of hyponatremia due to excessive water retention in the kidney[131]. In summary, in this study, there are several factors supporting the assumption that elevated levels of AVP or SIADH could be a possible cause for the high proportion of hyponatremia found on admission in these patients. However, recapitulating the main results from study IV, elevated levels of AVP were not a found in a cohort of patients very similar to the one described in this study who displayed a similar proportion of hyponatremia on admission. Again, the status of several hormones seems to be significant altered in severe COVID-19 and needs further investigation.

There may be several aetiologies producing the hypernatremia observed in Study III. Commonly, ICU-related hypernatremia is iatrogenic due to administration of sodium containing infusions and/or fluid withdrawal combined with dysregulation of autonomous processes necessary for water-and electrolyte homeostasis. However, irrespectively of the cause, ICU-hypernatremia is associated with poor outcome and should be avoided if possible [78]. In this study, the development of hypernatremia during the first two weeks of admission was associated with a markedly increased risk of death and increased length of hospital stay compared to patients not developing hypernatremia. In the patients admitted to the ICU in study III, the speed with which the hypernatremia developed following an initial hyponatremic state on admission was impressive, reaching peak vales on day 10 (Figure 9). However, the contribution of sodium substitution to this finding is unclear. Admission to the ICU due to critical illness usually prompts initiation of multi-organ support measures. This support ultimately affects electrolyte-and fluid balance. In ARDS, dehydration of the lung parenchyma is recommended in consensus guidelines[101, 132]. At the beginning of the pandemic these guidelines were upheld and may have contributed to the rapid increase in sodium observed in this study. However, the simultaneously decrease in hematocrit seen during the evolving hypernatremia suggests dehydration may not be the main cause of hypernatremia.

When considering the RAAS in this setting, the strong association between the RAAS and fluid-and electrolyte status are well described. The combined actions of ANG II on the proximal tubule and the adrenal cortex (release of aldosterone) will increase sodium reabsorption in exchange for H^+ and thus blood osmolarity will rise. Increased osmolarity will stimulate release of AVP with increased renal water reabsorption and consequently cause blood volume expansion. Aldosterone will further increase sodium reabsorption and osmolarity, in exchange for potassium. In study III, potassium levels were not assessed in the

ICU patients because the use of potassium replacement, which were used frequently, would have been a strong confounded factor. The administration of replacement therapy indicates potassium deficit, but this is not unique to severely ill COVID-19 patients since hypokalemia is a common finding in all ICU-patients. However, the prevalence of hypokalemia in COVID-19 patients has been found to be high and associated with poorer outcome[133]. Overall, these findings are complex, and the interpretations are difficult. Still, there was a clear association between hypernatremia, without signs of dehydration, and poorer outcome in patients with on-admission hyponatremia and potassium deficiency. This may be the effect of a viral induced RAAS-imbalance with direct and downstream effects of a classical RAAS-activation (Figure 11).

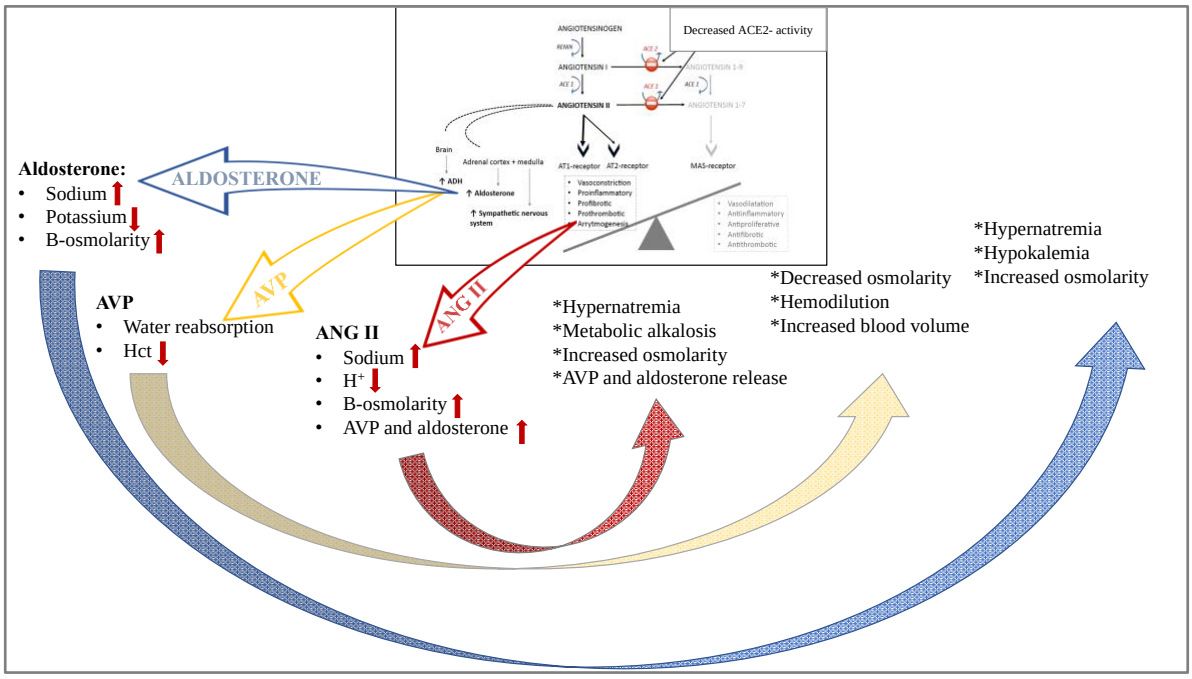

Figure 11. *Illustration of water-and electrolyte changes in a situation of classical RAAS activation due to insufficient ACE2 activity*.

Interestingly, a sudden AVP depletion, could further contribute to the rapid rise in S-Na (from an initial hyponatremic state) observed in patients with poorer outcomes (Figure 11). This association needs to be assessed in the coming future.

Summary of the overall findings in this thesis.

We found that unrestrained ANG II effects, explored in an experimental setting, were associated with a pathophysiological state sharing many features with severe COVID-19. Further, we found that, common to all included patient cohorts in Study II-IV, the presence of one or more previously known or unknown comorbidities within the spectra of the metabolic syndrome were significant. This finding has been echoed worldwide and pre-morbid metabolic status may be one of the most important determinants for COVID-19 severity. The further findings of altered biomarkers of the RAAS and the arginine vasopressin axis in patients with severe COVID-19 indicates a viral interference in the core of our physiology

with impact on several vital physiological processes. In vulnerable people, with a chronic activation of the classical RAAS-pathway, such viral interference may generate an acute on chronic state with manifestations of an aggravated metabolic syndrome, i.e., an acute on chronic metabolic syndrome (Figure 11).

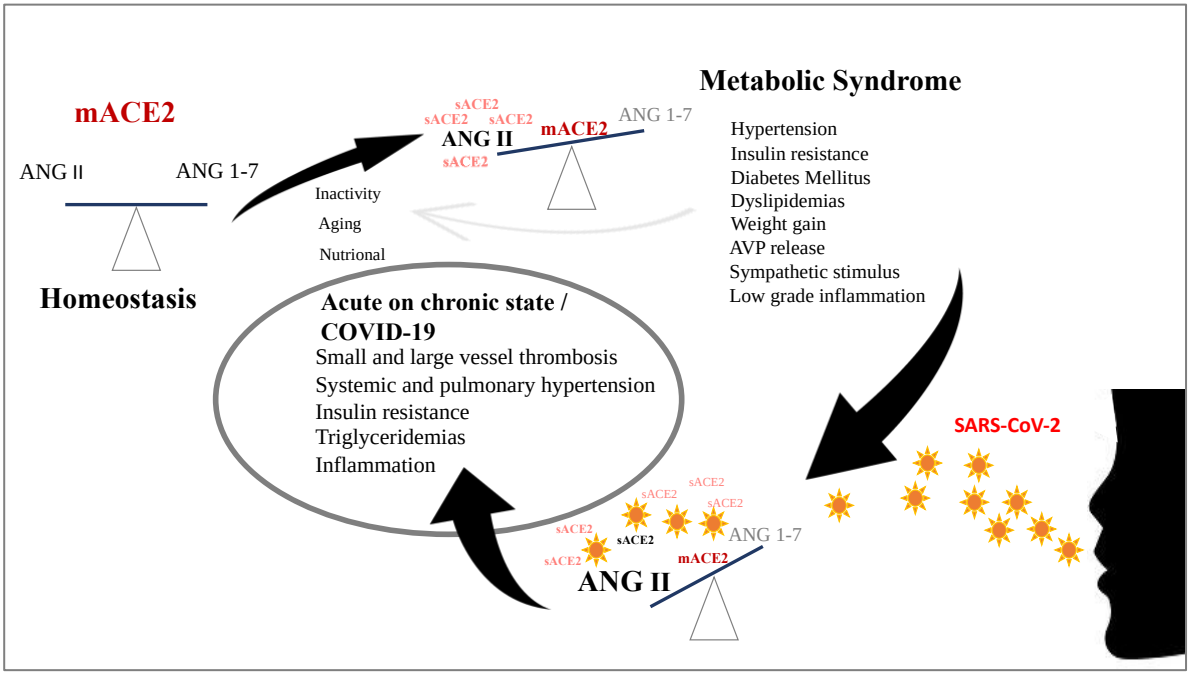

Figure 11. *Illustrations of how a SARS-CoV-2 interaction may generate a metabolic acute on chronic situation in patients with a pre-existing metabolic vulnerability. In a homeostatic state, the ACE2-levels are sufficient to maintain balance within the RAAS. Factors altering this balance such as age and an unhealthy way of living alters this balance, skewing the RAAS in favour of the classical pathway of RAAS, slowly increasing ANG II effects (e.g., development of the metabolic syndrome). Any further strain on the system with decreased ACE2-activity will accelerate the process and aggravate all symptoms of increased ANG II effects. ANG II= angiotensin II; ANG 1-7= angiotensin 1-7; mACE2=membrane bound angiotensin converting enzyme type 2, sACE= soluble angiotensin converting enzyme type 2; AVP= arginine vasopressin.*

6.1 LIMITATIONS

All studies in this thesis, except for study I, are observational cohort studies. In this thesis only adults were studied, and consequently, these results cannot be extrapolated into a pediatric population.

In study I, a large animal model was used to explore the effects of a pharmacologically induced RAAS-imbalance similar to that described in SARS-CoV-2. The animals were not infected with SARS-CoV-2 and previously healthy. The experimental part of study I, is hypothesis generating and thus have limited generaliseability. In addition, a retrospective radiologcal cohort of COVID-19 patients and clinical data from two COVID-19 ICU-patients

were used in a translational fashion. There were some losses of data because of the 339 scans initially included. In total, 50 scans were excluded due to technical errors and motion artifacts complicating or preventing analysis.

Study, II-IV are all conducted in a single center, limiting generalisability. However, the unique circumstances brought by the pandemic with striking homogeneity in the cohort of severe COVD-19 patients worldwide, regarding epidemiology, clinical presentation, co-morbidities, and outcome, increases the generalisability of our findings.

In study II, hospitalized SARS-CoV-2 positive patients with HbA1c values recorded within the prior of three months were included. However, HbA1c was not routinely analysed in all patients admitted to the study hospital and selection bias may therefore be present. In Study II, patients were excluded if they were readmitted to the ICU, displaying a SARS-CoV-2-negative PCR-test or if they had received extra corporeal membrane oxygenation (ECMO). Patients treated with ECMO were excluded because hemolysis frequently occurs during ECMO treatment, and this can lower blood HbA1c. In total, 86 patients were excluded from the study II. Another limitation in this study was the lack of information regarding whether patients had received a blood transfusion prior to HbA1c testing or not. This is an intervention which could result in falsely low values of the HbA1c test thus misclassification may be present but an error ought to be small. The confidence intervals in the analyses of chronic dysglycemia and HbA1c were wide. However, the point estimated for prediabetes, unknown diabetes and uncontrolled diabetes were also high indicating that a type I error (falsely positive finding) is unlikely.

In study III, hospitalised SARS-CoV-2 positive patients were included and identified in local registers. In all, 22 patients were excluded due to loss to follow-up. The lack of data regarding fluid input and output complicates the identification of the cause of the observed electrolyte disturbances. The presence of CRRT or any electrolyte substitutions are additional sources of error when interpreting the data.

In study IV, SARS-CoV-2 positive patients in need of intensive care were included and identified on admission to ICU. In addition, a control group including 10 gender-and age matched healthy, volunteers were included. They were identified in the hospital surroundings. PCR-SARS-CoV-2 testing was not performed on the volunteers however a thoroughly medical history was taken prior to inclusion. None of the controls were vaccinated against SARS-Cov-2. In this study, three patients were excluded due to technical errors in analyses. Factors complicating the interpretation of the results include different durations of COVID-19 disease prior to ICU-admission between individuals. The disease course and thus hormonal status may have differed between patients. Further, the short-lived nature *in vivo* of several of the included hormones makes analysis challenging. In addition, expanded analyses of the RAAS or hypothalamic-pituitary axis were not assessed which could have facilitated the interpretation.

7 CONCLUSIONS

From the studies comprising this thesis, the following conclusions have been drawn:

- In a translational fashion, pharmacological manipulation of the RAAS with activation of the classical RAAS leads to a pathophysiological state closely resembling the one found in severe COVID-19, including reduced lung perfusion and blood oxygenation, elevated pulmonary artery pressure, disturbed coagulation, alveolar damage and acute tubular necrosis.
- In hospitalised, SARS-CoV-2 positive patients with available HbA1c, the presence of unknown or poorly controlled dysglycemia was associated with a markedly elevated risk of respiratory failure.
- In hospitalised patients with severe COVID-19 hyponatremia on admssion was associated with increased risk of mechanical ventilation. In addition, in-hospital development of hypernatremia was associated with an increased risk of death.
- In critically ill COVID-19 patients admitted to the ICU, the levels of circulating ACE2 and copeptin (vasopressin) were low compared to healthy controls.This was not combined with low concentrations of circulating ANG-1-7 or hypotensiom. The levels of ANG II were preserved.

8 POINTS OF PERSPECTIVE

In this thesis we have shown that many of the characteristics in severe COVID-19 can be reproduced in an ANG II-rich environment as presented in study I. Another important finding in this thesis is the overrepresentation of patients with co-morbidities within the metabolic syndrome suffering from severe COVID-19. The link between several of these co-morbidities and the RAAS has been well established and treatment with RAAS-inhibitors improve metabolic status and prevents disease deterioration. Consequently, a prerequisite for cardiovascular health is a balanced RAAS which highlights the importance of an unstressed ACE2 status. However, a diminishing ACE2 activity may evolve silently and over time with a reciprocal slow increase in ANG II prior to manifest morbidity. In a situation like this, any further significant stress on ACE2 status, i.e., SARS-CoV-2 infection, will accelerate the process and generate an acute on chronic situation. This is problematic and a major health problem in an era where the metabolic syndrome constitutes a pandemic itself, with a significant number of people at risk of severe COVID-19 as well as of cardiovascular morbidity and mortality.

Another important finding in this thesis was the high proportion of patients with unknown borderline disease (prediabetes) or unknown manifest disease (undiagnosed diabetes) suffering from severe COVID-19. In the near future, early identification of people at risk for cardiovascular morbidity may be prioritised for improved public health. Pending additional screening tools for long-term status of the major constituents of the metabolic syndrome, HbA1c may be used as a surrogate to evaluate metabolic status consecutively. This testing may not be restricted to adults but could be used in younger individuals with risk-profile as well. In a future perspective, one of the most important take-home-message from this pandemic may well originate in the SARS-CoV-2-ACE2 interaction unmasking people at risk of future cardiovascular diseases.

Finally, regarding treatment options in COVID-19. With the pandemic still ongoing, no cure for severe COVID-19 has yet been found. Vaccination has markedly reduced the number of patients deteriorating to severe COVID-19 requiring of ICU-care. However, if the hypothesis that COVID-19 is an acute on chronic manifestation of the metabolic syndrome is correct, perhaps intensified treatment with the same drugs used in the treatment of the diseases of the metabolic syndrome (including angiotensin receptor blockers, anticoagulants, anti-diabetic preparations, and lipid-lowering formulas) should be initiated or continued. Ideally, this treatment regime should be instituted early in the infection. This may not cure, but hopefully dampen the response.

> "*Blowing out the candles on a birthday cake and then eat it may now be considered a pre-pandemic luxury."*
>
> *Susanne Rysz*

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First of all, I would like to send a thought to all the people that have suffered from severe COVID-19, either as a patient or as a relative. Some of those patients are anonymously represented in these statistics and interpretations. I sincerely hope that this thesis is a small step to a deeper understanding of the disease and something that may improve future care for these patients.

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