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THE ROLE OF ANGIOTENSIN CONVERTING ENZYME INSERTION/DELETION GENETIC POLYMORPHISM IN THE RISK, SEVERITY AND PROGNOSIS OF COVID-19 INFECTION

by
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ABSTRACT
OF THE THESIS OF

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Title: The Role of Angiotensin Converting Enzyme Insertion/Deletion Genetic Polymorphism in the Risk, Severity and Prognosis of COVID-19 Infection

Background: COVID-19 pandemic got the world's attention since the beginning of 2020. It has been observed that infected individuals present with different symptoms of varying severity; in addition, not all individuals get infected despite exposure. Risk factors such as age, sex and comorbidities play a major role in this variability; however, genetics may also be important in driving the differences in the incidence and severity of the disease. An Insertion/Deletion (I/D) polymorphism in the ACE-I gene may explain these genetic differences. The aim of this study is to determine whether the ACE I/D genetic polymorphism can be used as a marker of risk, severity and prognosis of COVID-19 infections.

Methods: More than 350 Lebanese subjects presented to AUBMC for COVID-19 PCR testing were recruited, and results are reported in this thesis on 266 subjects (142 cases and 124 controls), for whom clinical data and genotyping are complete. Clinical data were collected via filling a questionnaire and accessing the medical records. Peripheral blood was withdrawn for DNA isolation and *ACE* genotyping by standard PCR followed by band visualization on an agarose gel.

Results: The frequency of the *D* allele was most common (69%), which is congruent with previously reported literature in Middle Easterners. We showed that almost all previously reported factors and comorbidities also predict disease susceptibility and severity in the Lebanese. We also found a positive correlation between the ACE1 *I*-allele and the risk of contracting the COVID-19 disease. More specifically, the frequency of *II* genotype was significantly highest in cases compared to controls with individuals with the *II* genotype having a higher risk (OR=2.373) for contracting the COVID-19 disease. These results confirm Delanghe et al.'s simulations, and to our knowledge, we are the first to evaluate such an association in patients. As for disease severity, our results are in agreement with the literature whereby *DD* vs. *II+DI* was significantly higher in cases with the severe disease when compared to mild and moderate disease (P=0.003), hospitalized compared to non-hospitalized cases (P=0.027), and hypoxic compared to non-hypoxic cases (P=0.088). These results translate into the fact that subjects with *DD* genotype have a higher probability of experiencing severe COVID-19 symptoms (OR=7.173), to be hospitalized (OR=3.398), and/or to be hypoxic (OR=4.735).

Conclusion: These preliminary results show a positive correlation between the *ACE1 I*-allele and the risk of contracting the COVID-19 disease, and between *ACE1 D*-allele and worse outcome of the COVID-19 infection. As such, genotyping for *ACE1 I/D* in parallel to COVID-19 testing could be used to elicit the disease risk and severity for better prognosis and management.

TABLE OF CONTENTS

ILLUSTRATIONS

Figure

TABLES

Table

ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ACE2	Angiotensin Converting Enzyme 2
ACE-I	Angiotensin Converting Enzyme Inhibitors
ACS	Acute Coronary Syndrome
AF	Allele Frequency
Ang II	Angiotensin II
Ang(1-7)	Angiotensin (1-7)
APN	Aminopeptidase N
ARB	Angiotensin Receptor Blocker
ARDS	Acute Respiratory Distress Syndrome
AT	Anti-thrombin
AT1R	Angiotensin II Type 1 Receptor
AT2R	Angiotensin II Type 2 Receptor
AT4R	Angiotensin Type 4 Receptor
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CFH	Cell-Free Hemoglobin
CI	Confidence Interval
CKD	Chronic Kidney Disease
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Disease 2019
CRP	C-Reactive Protein
CT	Cycle Threshold
CVD	Cardiovascular Diseases
D/D	Deletion/Deletion
DIC	Disseminated Intravascular Coagulation
DNA	Deoxyribonucleic Acid
DPP4	Dipeptidyl Peptidase 4
EF	Ejection Fraction
EQTL	Expression Quantitative Trait Loci
ERGIC	Endoplasmic Reticulum-Golgi Intermediate Compartment
FDP	Fibrinogen Degradation Products
FIB	Fibrinogen
GTEx	Genotype Tissue Expression
HCoVs	Human Coronaviruses
HDL	High Density Lipoprotein
HTN	Hypertension
I/D	Insertion/Deletion
I/I	Insertion/Insertion
ICU	Intensive Care Unit
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
IRB	Institutional Review Board
LDL	Low Density Lipoprotein
LRT	Lower Respiratory Tract

MERS	Middle East Respiratory Syndrome
MS	Multiple Sclerosis
NIV	Non-Invasive Ventilation
NK	Natural Killer cells
NSP	Non-Structural Proteins
OR	Odds Ratio
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PP	Polyproteins
RAS	Renin-Angiotensin System
RBD	Receptor Binding Domain
RNA	Ribonucleic Acid
RT	Respiratory Tract
RTC	Replication and Transcription Complex
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-1	Severe Acute Respiratory Syndrome Coronavirus 1
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
TIV	Trivalent Inactivated Seasonal Influenza Vaccine
TMPRSS2	Transmembrane Protease Serine 2
TRS	Transcription Regulatory Sequences
URT	Upper Respiratory Tract
VTE	Venous Thromboembolism
VWF	Von Willebrand Factor
WHO	World Health Organization

CHAPTER I

INTRODUCTION

A. Coronavirus

1. History

Coronaviruses are a family of viruses that cause intestinal and respiratory illnesses in humans and animals. The subgroups of the coronavirus's family are alpha, beta, gamma and delta coronaviruses [1] [2]. Till today, there are seven human coronaviruses (HCoVs) identified, all of which fall into the genera of alpha and beta coronaviruses [3] [4] (Table 1). HCoV-NL63, HCoV-229E, HCoV-OC43 and HKU1 usually cause mild upper respiratory diseases and sometimes they can lead to severe infections in the young and the elderly [5] [6]. With the appearance of severe acute respiratory syndrome (SARS) in China in 2002 and Middle East respiratory syndrome (MERS) on the Arabian Peninsula in 2012, it was shown that these two are highly pathogenic viruses and cause severe respiratory symptoms in humans [5] [6]. All of the mentioned human coronaviruses are of animal origins: HCoV-NL63, HCoV-229E, SARS-CoV and MERS-CoV are of bat origin, while HCoV-OC43 and HKU1 are of rodent origin [5] [6].

Human coronavirus name	Illness
SARS-CoV-2	COVID-19
SARS-CoV	Severe acute respiratory syndrome (SARS)
MERS-CoV	Middle East respiratory syndrome (MERS)
HCoV-NL63	Usually mild respiratory illness
HCoV-229E	
HCoV-OC43	
HKU1	

Table 1 The different types of human coronaviruses [4].

2. Description

Coronavirus is a positive sense, single-stranded RNA genome virus [7]. It consists of structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N) [8] (**Figure 1**). Having an envelope makes it able to withstand mutations that can interrupt its infectious cycle. Coronaviruses have one of the largest known genomes consisting of 27 to 32 kb length which is more than double the length of the average RNA virus genome [3]. Coronaviruses have a spherical shape and the spikes they have are proteins that help the virus bind to cells. When seen under the microscope, the spikes of coronaviruses look like a crown, and corona is the Latin for ‘crown’. Detergents and alcohol disrupt the layer of membrane that is found underneath the spikes, which is why soap and hand sanitizer gels are effective against the virus [4]. Coronaviruses own the capacity of proofreading during replication which allows them to have lower mutation rates when compared to other RNA viruses [9].

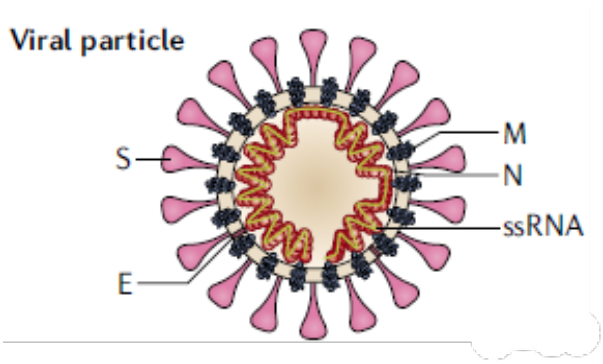

Figure 1 The coronavirus virion [8].

3. Viral Cycle

The virus's life cycle has 5 important steps: attachment, penetration, biosynthesis, maturation, and release. It starts when the virus binds to the host receptor (attachment), followed by entering the host cells either through endocytosis or membrane fusion (penetration). When there is release of contents of the virus inside the host cell, the viral RNA enters the host nucleus for replication. This viral mRNA is used to synthesize new viral proteins (biosynthesis) and finally new viral particles are made (maturation) and released [10].

a. Attachment and Penetration

The first and most important step of coronavirus infection includes the specific binding of the coronavirus spike protein (S) to the cellular receptors of the host. Several of these receptors have already been identified and include human aminopeptidase N (APN) for HCoV-229E, angiotensin-converting enzyme 2 (ACE2) for HCoV-NL63, SARS-CoV and SARS-CoV-2 and dipeptidyl peptidase 4 (DPP4) for MERS-CoV [8]. The coronavirus S proteins are divided into two functionally different parts: S1 is the surface-exposed domain which includes the receptors-binding domain (RBD) that is specific for binding to the host cell receptor, while the S2 domain consists of the heptad

repeat regions and the fusion peptide which promote the fusion of viral and cellular membranes [8]. After the outbreak of SARS-CoV in 2002, ACE2 was identified as the receptor which is necessary for infection by SARS-CoV, and the high genomic and structural similarity between the S proteins of SARS-CoV and SARS-CoV-2 confirmed ACE2 to be the receptor for SARS-CoV-2 [8]. When the S protein binds to the cellular receptors (ACE2) along with host factors (cell surface serine protease TMPRSS2) it promotes viral uptake and fusion at the cellular or endosomal membrane [8] (**Figure 2**). Between the S protein of SARS-CoV-2 and ACE2 is a unique salt-bridge interaction which allows the enhancement of binding affinity [3]. A structural analysis done by Chen et al. [11], showed that S protein of SARS-CoV-2 binds to ACE2. ACE2 is highly expressed in the lung, heart, ileum, kidney and bladder [12].

Spike (S) protein, a transmembrane trimeric glycoprotein, protrudes out of the viral surface [10]. When the spike binds to the host receptor, it undergoes a protease cleavage that includes 2 steps: cleavage at the S1/S2 cleavage site for priming and activation cleavage at the S2 site [13] [14] [15]. After the cleavage, S1 and S2 remain non-covalently bound, and the S1 stabilizes the membrane bound S2 subunit at the perfusion state [16]. Meanwhile the cleavage at the S2 site activates the spike for fusion by irreversible conformational changes [10].

A distinctive feature of SARS-CoV-2 is the existence of furin cleavage site at the S1/S2 site [10]. The S1/S2 site is also subjected to cleavage by transmembrane protease serine 2 (TMPRSS2) and cathepsin L [15] [17], but the presence of furin is responsible for the pathogenicity of the virus [10].

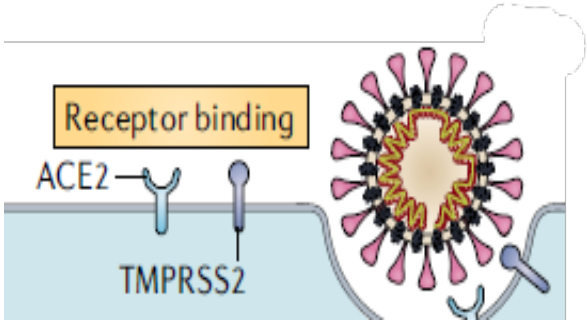

Figure 2 Viral entry through ACE2 and TMPRSS2 [8].

b. Biosynthesis: Genome Translation

Coronaviruses have remarkably large RNA genomes with 5′ and 3′ untranslated regions. At the 5′ end, there are two large open reading frames, ORF1a and ORF1b. ORF1a and ORF1b undergo translation and produce two polyproteins, pp1a and pp1ab, respectively. Both pp1a and pp1ab produce post-translationally 16 non-structural proteins: nsp1-nsp16. Proteolytic release of nsp1 occurs rapidly and allows nsp1 to target the host cell machinery [8]. Nsp2-11 supports viral replication and transcription complex (RTC) functions by modulating intracellular membranes, host immune evasion, and providing cofactors for replication. RTCs are found in convoluted membrane structures that are derived from the rough endoplasmic reticulum and anchored in place by viral transmembrane proteins [3]. On the other hand, nsp12-16 are responsible for the viral RTC and also for RNA synthesis, RNA proofreading and RNA modification [18] [19]. Most importantly, nsp14 is responsible for the 3'-5' exonuclease activity, allowing the viral RNA to have a unique RNA proofreading function [8] (**Table 2**).

Nonstructural Protein (nsp)	Function
nsp 1 & 3	Inhibition of IFN signaling and blocking of host innate immune response by promotion of cellular degradation and blocks translation of host's RNA
nsp 2	Binding to prohibition protein
nsp3 & 5	Promoting cytokine expression and cleavage of viral polyprotein
nsp 4 & 6	Contribute to structure of DMVs as transmembrane scaffold protein (DMVs formation)
nsp 7/8 complex	Processivity clamp for RNA polymerase by arms hexadecameric complex
nsp9	RNA binding protein phosphatase
nsp 10, 16 & 14	Stimulation of ExoN and 2-O-MT activity
nsp 12	Replication enzyme (RNA-dependent RNA polymerase)
nsp 13	RNA helicase, 5′ triphosphatase
nsp 14	Proofreading of viral genome
nsp 15	Viral endoribonuclease and chymotrypsin-like protease
nsp 16	Avoiding MDA5 recognition and inhibit innate immunity regulation

Table 2 Nonstructural proteins of coronaviruses and their function [20].

c. Biosynthesis: RNA Synthesis

Replication of the viral genome begins with synthesizing full-length negative sense genomic copies that become templates for the generation of new positive-sense genomic RNA [8]. Capping of the 5’ end of the viral mRNA occurs cotranscriptionally in the nucleus, and it is considered very important for the viral mRNA stability, translation initiation, and escape from the host innate immune system [3]. These new genomes are used for translation in order to generate more nsps and RTCs.

It was proposed by Sawicki and Sawicki [21] that coronaviruses have a distinctive feature of discontinuous viral transcription process that produces a set of 3’ and 5’ co-terminal subgenomic RNAs. During the synthesis of negative-strand RNA, the RTC interrupts transcription when it comes across transcription regulatory sequences (TRSs) that are located upstream of open reading frames (ORFs) in the 3’ end of the viral genome [8]. The discontinuous step during RNA synthesis allows an interaction between complementary transcription regulatory sequences (TRSs) of newly synthesized negative strand RNA and the positive strand genomic RNA [8]

The biogenesis of replication organelles starts with the interaction of nsps with the host cell factors during the replication cycle [8]. Replication organelles prevent the exposure of viral replication intermediates to cytosolic innate immune sensors [8].

d. Maturation and Release

After the translation of subgenomic proteins into structural proteins (for example, M, S and E), these move into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) [20]. The M protein is important for the protein-protein interactions needed for the assembly of coronaviruses, unlike the S protein, which is not required for the assembly, but its ability to interact with the M protein allows it to be incorporated into the virions [21]. The nucleocapsids (N) go into the cytoplasm where they assemble joining the ERGIC and forming a mature virion in the golgi vesicle [20] [23]. The M protein can bind to the nucleocapsid N and lead to the completion of the viral assembly [22]. The mature virion finally leaves the infected cell by exocytosis [23].

B. COVID-19

Towards the end of 2019, a new coronavirus (SARS-CoV-2) has been identified. At the beginning of 2020 the World Health Organization (WHO) named the disease COVID-19, that stands for coronavirus 2019 [4] [24].

1. Epidemiology

In December 2019, the very first case of COVID-19 was found in Wuhan, China. After that, it rapidly spread to other parts of the world becoming a global pandemic by

the beginning of 2020. According to the Lebanese Ministry of Public Health, it reached the Middle East, specifically Lebanon, on 21 February 2020. According to the Worldometer till 9 March 2021, there were over 100 million confirmed cases, with more than 2.5 million deaths [25].

2. Mechanism of Spread

An epidemiologic investigation was initially done in Wuhan, China, and associated with a seafood market that sells live animals. However, the virus became a pandemic through person-to-person spread [24]. The major challenge in the spread of this virus is that asymptomatic and presymptomatic people are infectious, 1 to 3 days prior to the onset of symptoms. There are two modes of viral transmission: direct and indirect (**Figure 3**).

a. Direct Transmission

The primary means of transmission of SARS-CoV-2 is direct person-to-person respiratory transmission [26]. The virus is released in respiratory secretions when an infected person coughs, sneezes, or talks, and this can infect other people if it is inhaled or makes direct contact with the mucous membrane [24]. The exposure and risk of transmission are increased if the infected person is within close proximity (1-m length) to the susceptible host [26]. Nevertheless, the risk of getting infected from an asymptomatic individual is less than that of a symptomatic individual. In an analysis done in Singapore, it was found that the risk of secondary infection was 3.85 times higher in people who contacted symptomatic individuals compared with people who contacted asymptomatic individuals [27].

i. Airborne Transmission

Airborne transmission occurs via aerosols, which are particles less than 100 μm in diameter [28]. An aerosol formation can occur during surgical and dental procedures, they can even be formed as droplets when a person is talking, sneezing or coughing [26]. Li et al. [29], suggested that the postoperative cough training respiratory exercise can produce a large amount of droplets and aerosols in the surrounding area. It is important to mention that dentists are at a higher risk of exposure as their patients are asked to gargle after oral procedures like drilling, extraction and drainage of dental abscesses [26].

ii. Body Fluids and Secretions

Other body fluids and secretions include saliva, urine, semen and tears [26]. In a study done by Azzi et al. [30], the respiratory swabs of two patients showed negative results while their salivary samples proved positive on the same day. The reason is that the virus can easily travel from the nasopharynx, but may still be present in the oral cavity since the epithelial cells of the oral mucosa highly express ACE2 receptors [31].

iii. Mother-to-Child Transmission

In a study done by Yu et al. [32], the authors found that one out of seven neonates tested positive for COVID-19 after 36 hours of birth. However, in two other studies by Khan et al. [33] and Li et al. [29], all the neonates born to 14 pregnant women tested negative for the virus indicating that transmission from mother-to-child might be rare though not completely absent. Newborns might also be infected due to

breastfeeding or due to inhalation of droplets produced by infected parents or healthcare professionals [26].

iv. Gastrointestinal Tract

Another mode of transmission is the gastrointestinal tract. For example, Xing et al. [34] studied stools, and they found that the viral shedding in the stools occurred even after the resolution of the symptoms and radiological findings. In contrast, samples from the nasopharynx and oropharynx were found negative for viral nucleic acid. Another study by Fan et al. [35] reported that an infant with COVID-19 continued to test positive in the anal swabs even after 14 days of testing negative by nasopharyngeal swab.

b. Indirect Transmission

Indirect transmission occurs via fomites or contaminated surfaces such as furniture found within an infected person's immediate environment or via objects used by the infected person such as the thermometer [26]. As a matter of fact, it is known that SARS-CoV-2 remains viable for days on smooth surfaces such as stainless steel, plastic and glass, allowing the transfer of an infection from contaminated surfaces to the mucosa of the eyes, nose and mouth via unwashed hands [9] [24]. In addition, in a study done in Singapore, viral RNA was detected all over the surfaces in the airborne infection isolation room of a patient with mild symptoms of COVID-19 before routine cleaning of the room [36].

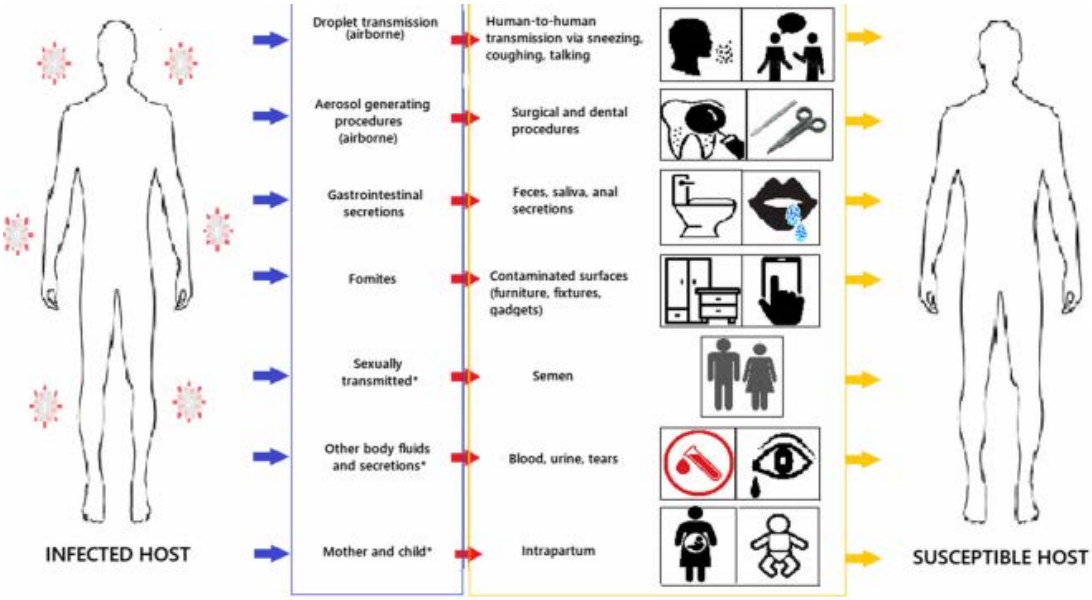

Figure 3 Modes of Transmission of COVID-19 [26].

3. Pathophysiology

COVID-19 disease is characterized by three phases: viral phase, inflammatory phase and pro-coagulant phase, and the stages of clinical symptoms of patients infected with SARS-CoV-2 widely range depending on the phases they go through (**Figure 4**).

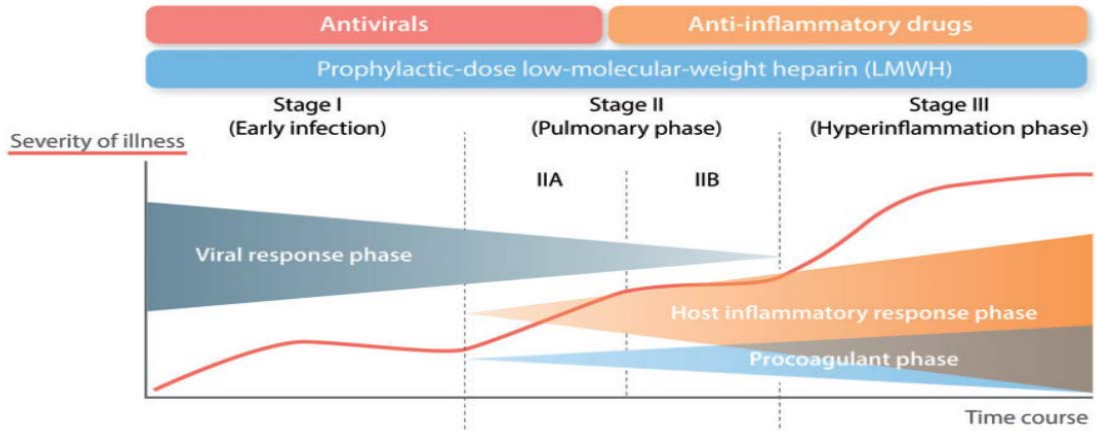

Figure 4 Phases and clinical stages of COVID-19. Adapted from Siddiqi et al. [37].

a. Phase 1: Viral response phase

In COVID-19 infection, viral loads are usually at their peak just before or at the symptom's onset [38]. Using reverse transcription polymerase chain reaction (RT-PCR), it is possible to test for an active virus infection since it detects the viral ribonucleic acid (RNA) that is shed at different sites throughout the body [39]. With the RT-PCR test, the cycle threshold (Ct) represents the number of replication cycles needed to produce a fluorescent signal, whereby lower Ct values represent a higher viral RNA load [38] (**Figure 5**).

Mallett et al. [39] showed that during the first 4 days of symptoms onset, 89% of nasopharyngeal tests were positive for COVID-19, however 10 days after the symptom's onset, the chance for testing positive for COVID-19 is diminished. It is important to mention that the upper respiratory tract (URT) sites are cleared of the virus much faster than the lower respiratory tract (LRT) sites, which means that lower rates of sample positivity are seen from URT sites [39]. Mallett et al. [39] also found that 39 out of 89 participants had a shorter detection duration through fecal samples than respiratory tract (RT) samples. Therefore, in order to have an early detection of the COVID-19, it is better to start testing by using respiratory sampling, specifically upper respiratory tract, while faecal sampling may be used to detect viral clearance as it is of no use during the early stages [39].

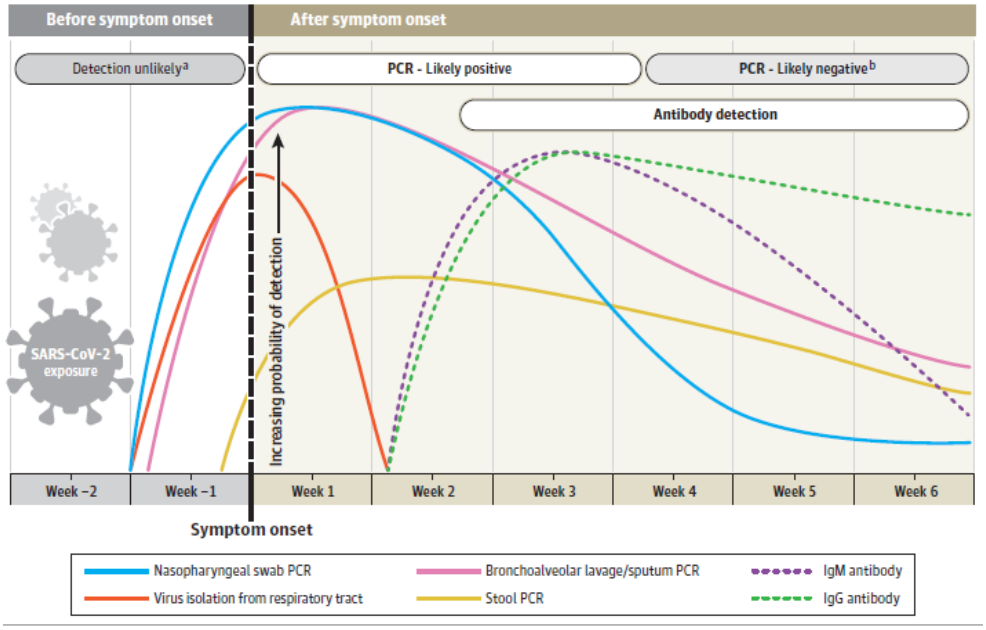

Figure 5 Estimated variation over time in RT-PCR diagnostic tests to detect SARS-CoV-2 infection relative to symptom onset [38].

b. Phase 2: Inflammatory response phase

i. Immune System

There are three main components for the innate immunity in the airway: epithelial cells, alveolar macrophages and dendritic cells [10]. Both dendritic cells and macrophages fight against viruses as innate immune cells until the adaptive immunity is involved [40]. T cell mediated responses against coronaviruses are initiated by presenting antigens through dendritic cells and macrophages [10]. Both dendritic cells and macrophages are known to be able to phagocytize virus-infected apoptotic epithelial cells thus leading to antigen presentation to T cells [10]. The antigen-presenting cells move to the lymph nodes to present viral antigens to T cells. $CD4^+$ T cells activate B cells to ensure the production of virus-specific antibodies, while the $CD8^+$ T cells have the capability to kill virally infected cells [10]. It has been reported that patients with severe diseases showed lymphopenia, specifically a reduction in peripheral blood T

cells, and increased plasma concentrations of proinflammatory cytokines [41] [42]. The more severe the conditions patients were in, the higher their IL-6 levels were, also $CD4^+$ and $CD8^+$ T cells were activated in those patients, and exhaustion of T cells leads to the progression of the disease [43] [41] [42]. In patients with severe COVID-19 infection, there is infiltration of a large number of innate and adaptive inflammatory immune cells in the lungs [10].

ii. Antibodies

It is possible to detect COVID-19 infection by measuring the host immune response to SARS-CoV-2 infection [38]. It is most important for patients who have experienced mild to moderate illness, during which the total antibody levels begin to increase starting from the second week of symptom onset [38] (**Figure 5**). The expected antibody response is to observe an early increase in IgM followed by a rise in IgG levels [44] [45] (**Figure 6**). However, serum IgG levels can also be seen at high levels either at the same time or earlier than IgM during SARS-CoV-2 [46]. SARS-CoV-2 virus-specific IgG and IgM reach a peak 17-19 days and 20-22 days after symptom onset, respectively [44]. When studying the seroconversion in infected patients, it was found that a higher percentage of patients showed earlier IgG seroconversion than IgM seroconversion [47]. In a study by Guo et al. [48], it took 5 days (range 3-6 days) for specific IgM and IgA antibodies to develop, and 14 days (range 10-18 days) for specific IgG to develop after the symptom's onset. On the other hand, To et al. [47] and Xiang et al. [49] found that seroconversion of IgM and IgG in patients infected with COVID-19 takes place between the third and fourth week of clinical illness. By the 5^{th} week, IgM

starts to decrease reaching lower levels and diminishes by the 7th week; however IgG still shows high levels even after the 7th week [50] (**Figure 5**).

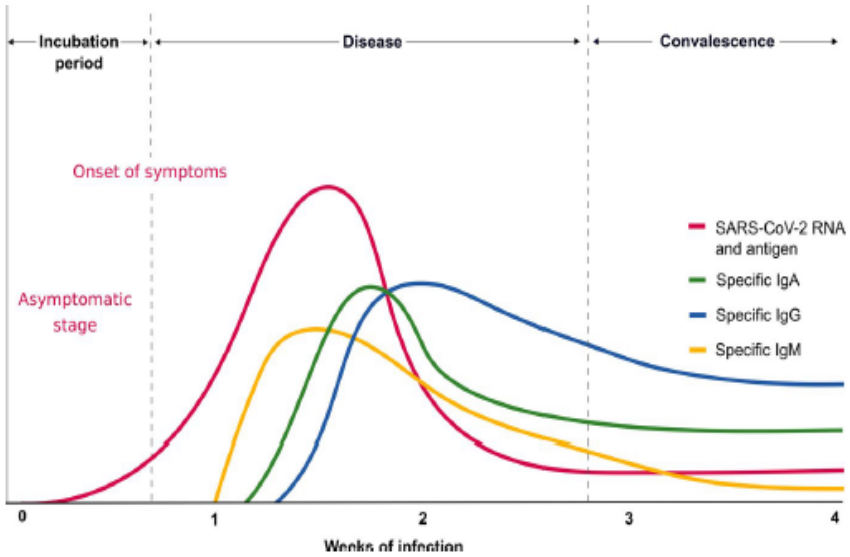

Figure 6 Specific antibody response to SARS-CoV-2 [44].

iii. Cytokine Storm

Similar to SARS and MERS, cytokine storm is a common feature in severe COVID-19 cases and a major reason for ARDS and multiorgan failure.

Cytokine storm is a complex network of several molecular events that have a common clinical phenotype of systemic inflammation, multiorgan failure and hyper-ferritinemia [44]. Cytokine storm is mainly induced by the activation of large numbers of white blood cells, B cells, T cells, NK cells, macrophages, dendritic cells, neutrophils, monocytes and resident tissue cells such as epithelial and endothelial cells that release high amounts of pro-inflammatory cytokines [44]. Evidence suggests that the virus-activated “cytokine syndrome storm” is the major reason of mortality in COVID-19 cases. Also, the presence of lymphopenia and cytokine storm together play a

major role in the pathogenesis of severe COVID-19 [44]. Patients with fever have increased circulating cytokines that are tolerated in mild cases, yet they become a tissue damaging storm in patients with severe COVID infection [44].

c. Phase 3: Procoagulant phase

Other than respiratory symptoms, thrombosis and pulmonary embolism were reported in severe diseases along with elevated D-dimer and fibrinogen levels [10]. In a study by Han et al. [51], it was found that anti-thrombin (AT) levels were higher in healthy people, in contrast to the levels of D-dimer, fibrinogen degradation products (FDP) and fibrinogen (FIB) that were higher in COVID-19 patients. Coagulation is considered part of a physiological response because it has been shown to occur due to infections [52] [53] [54] [55]. Although coagulation causes serious illness, it is considered to have an immune function, thus potentially being a defense mechanism against critical infections [56]. Therefore, it is advisable to monitor D-dimer and FDP levels in patients with COVID-19 since both were found to be particularly predictive of infection progression [51]. These markers also project new onset thrombotic events with 25% of critically ill COVID-19 patients developing venous thromboembolism (VTE) [57], and less commonly so for arterial thrombosis [58].

4. Symptoms and Severity of COVID-19

According to the Centers for Disease Control and Prevention (CDC) people may start to experience COVID-19 symptoms 2-14 days after exposure to SARS-CoV-2. Most of the COVID-19 patients recover after mild and moderate disease within one week; however, some patients develop the severe disease in the second week such as

pneumonia followed by cytokine storm, ARDS, multi-organ failure and disseminated intravascular coagulation (DIC) within the third week of the disease [44].

a. Clinical Stage I: Mild

Stage I is classified as Early Infection by Siddiqi et al. [37], whereby most patients experience mild symptoms suggestive of upper respiratory tract infections. General initial symptoms include fever, chills, cough, nasal congestion, headache, and sore throat. Patients might also experience gastrointestinal symptoms such as nausea, vomiting and diarrhea. It was reported that many people have gastrointestinal symptoms before even having fever or lower respiratory tract signs [59]. Symptoms are also considered mild when there is loss of smell (anosmia) or loss of taste (ageusia) which are more common in women than men [60].

b. Clinical Stage II: Moderate

Siddiqi et al. [37] named this stage the Pulmonary phase (**Figure 4**), whereby the patient has inflammation in the lung [37]. A patient is considered to have moderate symptoms when radiographic or clinical proofs of lower respiratory tract disease show a blood oxygen saturation of 94% or higher [1]. According to the WHO clinical progression scale [61], patients are considered to have moderate disease if they are hospitalized with or without oxygen therapy by mask or nasal prongs.

c. Clinical Stage III: Severe

A patient's symptoms are considered severe when he has marked tachypnea, hypoxia and lung infiltrates [62] [63] [64]. ACE2 is highly expressed on the apical side

of lung epithelial cells in the alveolar space, which is why the virus enters and destroys them [10]. Respiratory failure, shock and multi-organ dysfunction or failure are considered among the symptoms of critical illness [1] (Figure 7). Lymphopenia is found to be common among people who are experiencing severe COVID-19 symptoms [65] [43] (**Figure 8**).

According to the WHO clinical progression scale [61], patients are considered to have severe disease when, during their hospitalization, they require oxygen by non-invasive ventilation (NIV) or high flow, intubation, mechanical ventilation, vasopressors and dialysis.

This stage is known as hyperinflammatory phase [37] (**Figure 4**), where the patient can suddenly develop ARDS. A critically ill individual might develop ARDS after 8-9 days from symptom onset [43]. Patients with ARDS have difficulty inhaling oxygen as their lungs are filled with fluid and cell-free hemoglobin (CFH) occupying most of the airspace [44]. In addition, infected people have further difficulty breathing because of systemic destruction of red blood cells, leading to oxidized iron ions in the ferric state leading to CFH [44]. Of note that, CFH contributes to the cytokine storm since it increases proinflammatory cytokine expression and paracellular permeability [44]. Pathogenesis of ARDS is described by the presence of endothelial damage and increased capillary endothelial cell permeability in the lungs that cause fluid leakage into the pulmonary parenchyma [120]. The endothelial cell gaps allow the passage of fluid, neutrophils and cytokines into the pulmonary parenchymal space [44]; it appears within minutes to hours after acute lung injury (**Figures 7 and 8**).

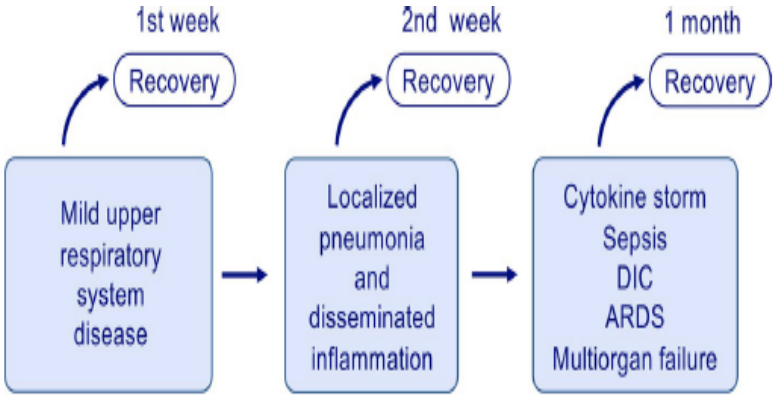

Figure 7 Different clinical stages of COVID-19. Adapted from Azkur et al. [44].

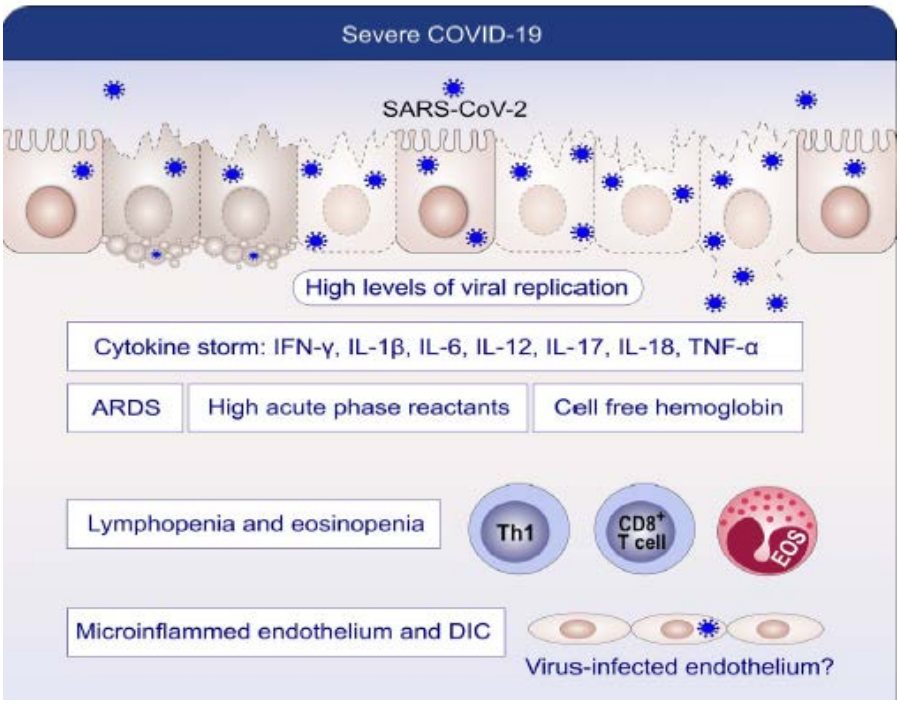

Figure 8 Pathogenesis of severe COVID-19 [44].

5. Renin-Angiotensin System and COVID-19

The renin-angiotensin system is a key factor for homeostasis since it regulates the blood pressure and electrolyte and water imbalance [66]. Following the cleavage of

angiotensinogen into angiotensin I by renin which is secreted by juxtaglomerular cells [67] [68], angiotensin I is further converted into angiotensin II by ACE. Angiotensin II works by activating one of the two pathways whose receptors, angiotensin II receptor type 1 (AT1R) and angiotensin II receptor type 2 (AT2R), have counteracting effects [69]. AT1R causes vasoconstriction, hypertension and promotes inflammation; however, Angiotensin II may further be converted into angiotensin IV that binds to AT4R causing thrombosis [70]. On the other hand, Angiotensin II may also bind to AT2R which has a protective and regenerating role in mediating vasodilation and natriuresis [71]. Angiotensin II, which is the key factor in achieving equilibrium, can also be inactivated by ACE2 and transformed into angiotensin 1-7 which in turn binds to Mas receptors leading to vasodilation and hypotension [70].

When the S protein of SARS-CoV-2 binds to ACE2 receptors, this leads to lower levels of ACE2 and lesser angiotensin II inactivation, thus inhibiting the ACE2/Ang-(1-7)/Mas receptor pathway and altering the balance of RAS [72]. This leads to overstimulation of AT1R and conversion of angiotensin II into angiotensin IV potentially further complicating the symptoms of COVID-19 by promoting thrombosis and vasoconstriction [70] (**Figure 9**).

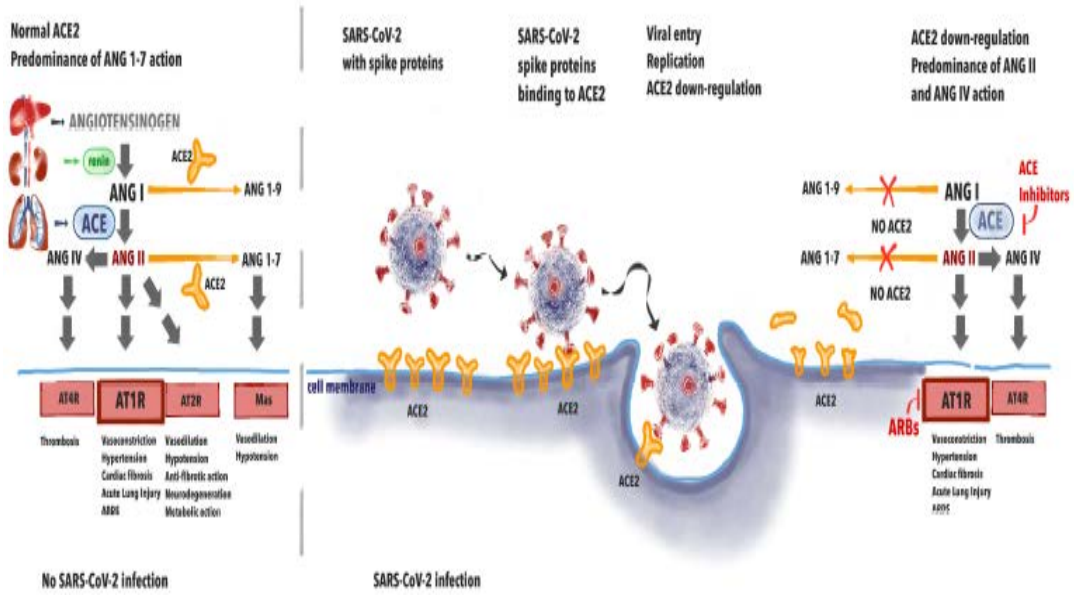

Figure 9 The interaction between the renin-angiotensin system and SARS-CoV-2 virus [70].

6. Risk Factors

Several risk factors play an important role in the extent of the severity of the infection and the risk of developing illness from COVID-19, with most being linked to ACE2 expression including comorbidities such as hypertension, diabetes, renal disease, and COPD.

a. Age

i. Children

According to the CDC, children with COVID-19 have mild symptoms or none at all; however, children with medical conditions may have an increased risk of severe illness and death from COVID-19. There is a difference between the severity of COVID-19 and the duration of the virus-shedding period, whereby children have fewer viral loads even after getting infected with COVID-19 [10]. A possibility could be due

to ACE2 expression. For instance, a previously done study by Jia et al. [73], showed that ACE2 is found more abundantly on well-differentiated ciliated epithelial cells, and since human lung and epithelial cells continue to develop after birth, ACE2 expression is lower in children [10]. Also, other viruses present in the lungs and airway mucosa, which are common in children, compete with SARS-CoV-2 and limit its growth [10]. In a youthful system, alveolar macrophages recognize the infected pneumocytes, release cytokines, and present antigens to T cells. T cells, in return, kill the infected cells to prevent the spread of the virus [74].

ii. Adults

It has been reported that older age is an independent predictor of mortality in SARS and MERS. With aging, there is an increase in proinflammatory cytokines responsible for the function of neutrophils related to the severity of ARDS [10]. With aging, the immune system undergoes immunosenescence which is a gradual decline in immune function, thus affecting pathogen recognition, alert signaling and clearance. A chronic increase in systemic inflammation also occurs, called inflammation, which arises from an overactive but ineffective alert system. Both immunosenescence and inflammaging are described as major drivers of the high mortality rates in older patients. In an aged system, a limited reserve of T cell receptors and defective macrophages is less effective thus more cells are infected leading to a greater viral replication. This allows the endothelial cell lining of the capillary to become inflamed, fibroblasts to become activated, and the viral components to enter the bloodstream leading to acute lung injury and ARDS [74]. With aging, there is continuous antigen stimulation and a shift from naïve T cells to central memory T cells due to thymic

involution. This process is accompanied by the loss of expression of co-stimulatory molecules, CD27 and CD28, with increased susceptibility to infections [10] (**Figure 10**).

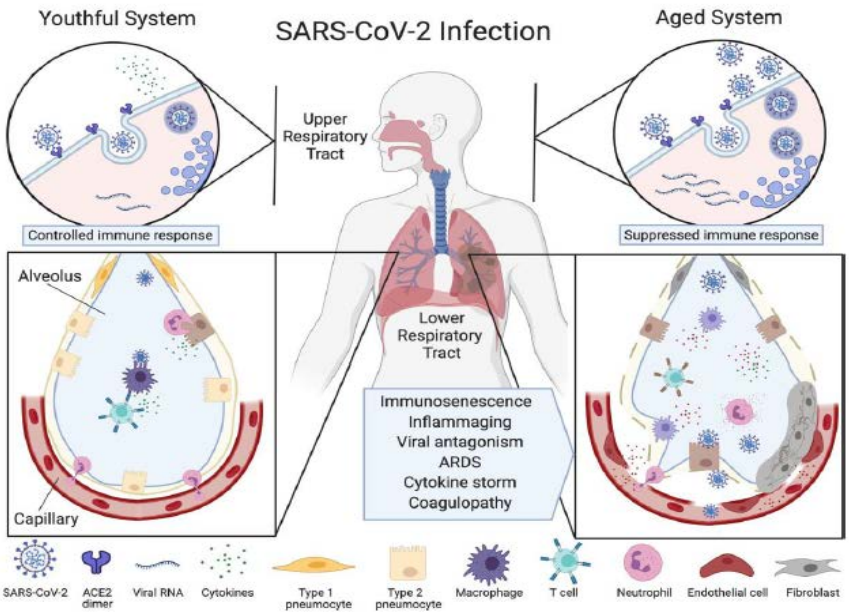

Figure 10 Ineffective clearance of SARS-CoV-2 infection in the aged respiratory system [74].

b. Sex

Males are more susceptible to SARS-CoV-2 than females for many reasons. First, the ACE2 receptor which has a very important role in the entry of the virus, is found on the X chromosome [75]. Second, the male sex hormone testosterone is known to suppress the immune system, while oestradiol protects women from infectious diseases by increasing T cell responses and antibody production. This suggests a potential protective effect of oestradiol against inflammatory immune responses associated with mortality in COVID-19 [76]. Third, there are sex differences in the innate and adaptive immune systems that may give the female an advantage in COVID-19. For instance, with respect to the adaptive immune system, females present higher

numbers of CD4+ T cells, more CD8+ T cell cytotoxic activity and increased B cell production of immunoglobin compared to males [76]. Fourth, the X chromosome contains many immune-related genes that may be variably expressed on both alleles in immune cells in females, which increases the immune response diversity. It has been reported that women achieve equivalent protective antibody titers to males at half the dose of trivalent inactivated seasonal influenza vaccination (TIV), and serum testosterone levels inversely correlate with TIV antibody titers [77]. Also, B cells in females produce more antigen-specific IgG in response to TIV [78]. All these findings show the increased capacity of females to achieve humoral immune responses compared to males [76]. Females also produce more type 1 interferon when toll-like receptor 7 senses a viral RNA than males, which is important for the early response in COVID-19.

c. Blood Group

When SARS-CoV-1 became an epidemic, Cheng et al. [79] suggested that blood groups are related to this disease whereby group O individuals were less susceptible. This trend was also seen in SARS-CoV-2 [80]. In a study by Li et al. [81], the percentage of group A infected individuals was much higher than in the healthy controls whereas group O was lower, but it is important to mention that group A individuals had more underlying comorbidities. It has been shown that anti-A was seen less in infected patients compared to those who lack anti-A [80]. Li et al. [81] showed a higher risk of hospitalization for blood group A SARS-CoV-2 infected individuals compared to those of blood group O that were at lower risk. In addition, and in a prospective observational study of critically ill patients with COVID-19 in Canada, the authors found that people with blood group A or AB had a prolonged intensive care unit admission and a higher

risk of requiring mechanical ventilation in comparison to blood group O or B patients [82]. Additionally, it was found that the anti-A antibodies in group B individuals were less protective than the anti-A antibodies in group O individuals, which is expected to be due to the higher levels of IgG anti-A and B in plasma of group O individuals [83]. Interestingly, Latz et al. [84], showed that individuals who have the Rhesus blood group had higher chances to test positive for SARS-CoV-2.

There have been few proposed mechanisms for the potential association between ABO blood groups and COVID-19 disease severity. The antigens of ABO blood groups are oligosaccharides found on red blood cells and other types of cells, allowing anti-A antibodies to bind to A antigens found on the viral envelope leading to prevention of infection of the target cells [80]. There has been a hypothesis, which was later proven by Guillon et al. [85], that the human anti-A antibody can bind to the S protein of SARS-CoV-2 thus preventing the interaction between ACE2 receptor and S protein [80]. Furthermore, it is notable that blood group A individuals have higher levels of Von Willebrand factor (VWF) and clotting factor VIII [80]. VWF is responsible for the protection of factor VIII from proteolytic degradation [86]; thus high levels of VWF lead to thrombosis [87] [88] (**Figure 11**).

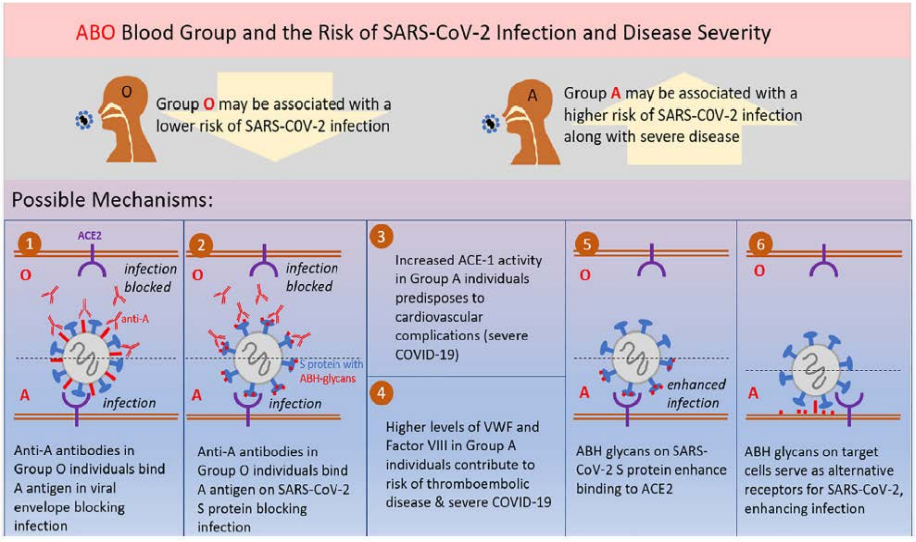

Figure 11 Graphical summary of proposed mechanisms for the association between ABO blood groups and SARS-CoV-2 infection [80].

d. Smoking

Cai et al. [89] evaluated the effect of smoking on the expression of pulmonary ACE2 and found that smoking causes bronchial epithelial cell remodeling along with hyperplasia of goblet cells where ACE2 gene is mainly expressed. Another recent study by Lukassen et al. [90] indicated that smokers have a risk of experiencing COVID-19 infection complications depending on their ACE2 expression profiles, contributing to differences in the susceptibility of infection, the severity of the disease and treatment outcome [89]. It was observed in a systematic review and meta-analysis that current smokers had a lower risk of being hospitalized with COVID-19 [169]. The studies also showed that current smokers had a higher risk of experiencing adverse outcomes when compared to non-smokers; however, when compared to former smokers they were less likely to have adverse outcomes [169]. The reason behind the higher risk of adverse outcomes in former smokers is still unclear.

Pulmonary cells have nicotinic receptors [91] [92] and nicotine upregulates the expression and/or activity of ACE [93]. For instance, in a study done by Cai et al. [89], it was found that ever smokers have an upregulation of ACE2 gene expression in the lungs, suggesting that smokers have an increased risk for SARS-CoV-2 entry and its binding to ACE2.

The effect of nicotine from smoking on ACE2 could be through angiotensin II and AT1R [94] (**Figure 12**). Oakes et al. [93] supported this, who found that nicotine increases the expression and/or activity of renin, ACE and AT1R through ACE/Angiotensin II/AT1R pathway, whereas it decreases the expression and/or activity of AT2R. Oakes et al. [93] also mentioned that nicotinic receptor activation leads to the cleavage and activation of S protein of SARS-CoV-2 through enhanced protease activation. Interestingly, furin was also found to be upregulated due to smoking but not to the extent of ACE2 [89].

Although it is very clear that smoking has its own side effects on the human body notwithstanding COVID-19; there are possible mechanisms through which smoking might be helpful in the course of COVID-19 disease. Usman et al. [174], discussed the possible interactions that could occur between smoking and COVID-19. Nicotine is considered to be protective by potentially inhibiting the production of pro-inflammatory cytokines without affecting the anti-inflammatory cytokines [175]. Moreover with smoking, nitric oxide is produced and it has the ability to inhibit the replication of SARS-CoV-2 inside the human cell [176]. Another possible mechanism could be the upregulation of ACE2 which is an anti-inflammatory protein [174].

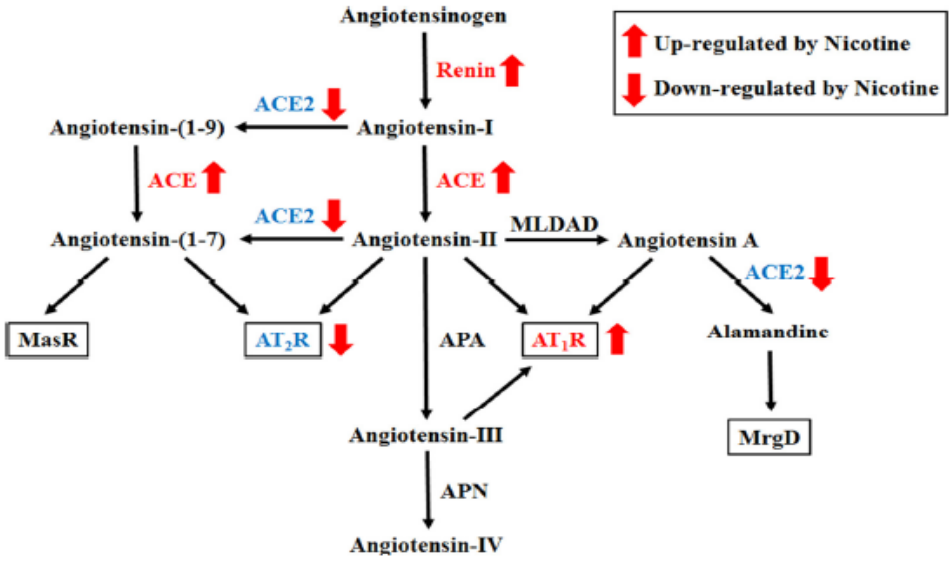

Figure 12 The interaction between the renin-angiotensin system and nicotine [93].

e. Comorbidities

Older patients or people of any age who have underlying medical conditions have even worse outcomes when infected with COVID-19. It was observed that these comorbidities also increase the chances of infection (**Figure 13**). The elderly with chronic health conditions such as diabetes, cardiovascular or lung disease are at higher risk of developing severe illness and at a higher risk of death if they get ill [95]. People with chronic obstructive pulmonary disease (COPD) or other respiratory illnesses are also at a higher risk for severe illness when infected with COVID-19 [95]. It is also important to mention that patients with asthma are at a disadvantage since the virus affects the respiratory tract, leading to more asthmatic attacks, pneumonia and acute respiratory distress [95]. Common comorbidities such as hypertension, diabetes, COPD, cardio-cerebrovascular disease and obesity were found to be more significant risk factors when compared with other underlying medical conditions [95]. The mechanisms behind these associations vary though the most compelling link is the increased expression of ACE2 as seen in **Table 3** below.

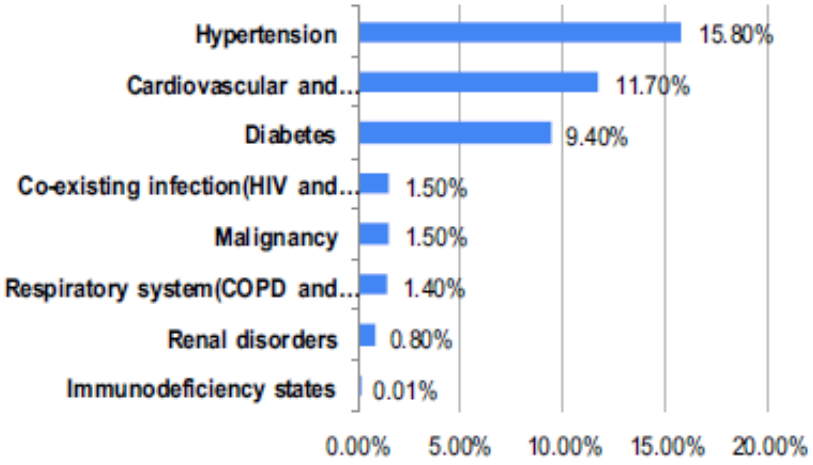

Figure 13 Comorbidities associated with COVID-19 infection [95].

S. No.	Disease	SARS-CoV-2 targets	Symptoms
1	Hypertension	Upregulate ACE-2 expression	Increased blood pressure with pneumonia
2	COPD	Upregulate ACE-2 expression	Severe hypoxemia
3	CVD	Impaired immune system	Myocardial injury, heart attack
4	Liver diseases	ACE-2 expression in liver cells, i.e., cholangiocytes, endothelial cells hepatocytes, and Kupffer cells	Elevated serum aminotransferases
5	Malignancy	Impaired immune system	Adult respiratory distress syndrome
6	Asthma	Delayed innate antiviral immune response and delayed secretion of IFN-λ	Chronic respiratory diseases along with pneumonia-like symptoms
7	Renal diseases	Increase secretion of enzymes, dipeptidyl peptidase-4 and angiotensin-converting enzyme (ACE-2)	Acute kidney injury (AKI)
8	HIV	Antiretroviral therapy (ART) with the impaired immune system and ACE-2 receptor in the lungs	Pneumonia like symptoms with jaundice
9	Obesity	The abnormal secretions of cytokines, adipokines, and interferons	Chronic low-grade inflammation of abdominal obesity with effect on bronchi and lung parenchyma
10	Diabetes	ACE-2 expression, impaired T-cell function and increased interleukin-6 (IL-6)	Pneumonia like symptoms

Table 3 Comorbidities, symptoms and targets concerning SARS-CoV-2 [97].

i. Diabetes

Patients with type 2 diabetes are more likely to have increased severity of COVID-19 [95]. A cohort study [96] showed that patients with type 2 diabetes needed increased interventions during their hospital stay versus non-diabetic patients, and those

with more inadequate blood glucose levels had an increased mortality rate than those with better glucose control. People with diabetes are more prone to get infections because of impaired phagocytic cell capabilities [97]. In addition, according to the Mendelian randomization analysis, an elevated level of ACE2 receptors is found to be related to diabetes [97]. Furthermore, furin, a proprotein convertase involved in the entry of the virus inside the host cell, is expressed in high levels in diabetic patients [97]. A dysregulated immune response together with increased ACE2 receptors and furin expression lead to a higher lung inflammation rate and lower insulin levels [97].

ii. Hypertension

A study done by Lippi et al. [98] stated that hypertension is a risk factor for developing severe COVID-19. Uncontrolled hypertension is associated with COVID-19 infection coupled with a high case fatality rate [97]. In a cohort study done by Qin et al. [99], the authors observed that 44% of the COVID-19 infected patients showed at least a single underlying disorder; however, a higher percentage of hypertension was seen in severe cases. The reason why patients with hypertension have a higher risk to develop severe symptoms of COVID-19 is unclear [100]; however, it is believed that when hypertension is treated with ACE inhibitors, this leads to the upregulation of ACE2 receptors which may help to get infected with COVID-19 [101].

iii. Obesity

Obesity is one of the less highlighted comorbidities though it is an important risk factor for COVID-19 disease severity. Obesity, defined as a body mass index (BMI) above 30kg/m2, is directly related to having a reduced oxygen saturation of

blood by compromised ventilation at the base of the lungs [97]. Moreover, Emilsson et al. [102] have observed that obese patients have higher levels of circulating ACE2 thus increasing the susceptibility of getting infected by SARS-CoV-2. Obesity may also be associated with low-grade inflammation, mainly due to abnormal secretions of cytokines, adipokines, and interferon, leading to a compromised immune response [97].

iv. Cardiovascular Disease (CVD) and Hypercoagulable State

It turns out that there is a bidirectional interaction between CVD, hypercoagulable state and COVID-19 infections. For instance, Huang et al. [43] and Wang et al. [103] found that CVD increased the risk of mortality in COVID-19 infected patients. In addition, COVID-19 was also found to facilitate the development of cardiovascular disorders such as arrhythmia, myocardial injury, acute coronary syndrome and venous thromboembolism [104] [105] [106] (**Figure 14**).

In a multicenter cohort study by Zhou et al. [107], 33 out of 191 patients with COVID-19 had acute cardiac injury, of whom 32 died. Another report done in Wuhan showed that 5 out of 41 patients with COVID-19 showed myocardial injury along with elevated troponin I levels, 4 of whom had to be admitted to the ICU [43].

It was found that COVID-19 can also activate acute coronary syndrome (ACS) [108]. The pathway leading to COVID-19 induced ACS might include plaque rupture, coronary spasm or microthrombi [109] [110]. Libby et al. [109] found that activated macrophages produce a tissue factor which is a procoagulant thus leads to the formation of thrombus when there is plaque rupture.

In a study involving 799 patients from Wuhan by Chen et al. [111], the authors found that heart failure was one of the most common observed complications of

COVID-19, with a high percentage of incidence of 49% in patients who died and 24% in all patients. In another study done by Mehra et al. [112], the authors found that elderly patients who have reduced diastolic function are prone to develop heart failure with preserved EF during COVID-19 infection.

Arrhythmias were observed by Guo et al. [105] to be an important symptom of COVID-19 in individuals who do not report fever or cough. Furthermore, a study done in Wuhan included 187 hospitalized patients with COVID-19 among which those who had increased levels of troponin T had higher chances to develop malignant arrhythmias [113].

As for hypercoagulation, De Rosa et al. [115] found that coagulation abnormalities were correlated with COVID-19 resulting in thromboembolic events (**Figure 14**). Individuals infected with COVID-19 were found to have elevated D-dimer levels, diminished platelet counts and extended prothrombin time [114]. Panigada et al. [116] and Ranucci et al. [117] observed that patients infected with COVID-19 have increasing fibrinogen and factor VIII levels, which are among the reason behind the occurrence of the hypercoagulable state.

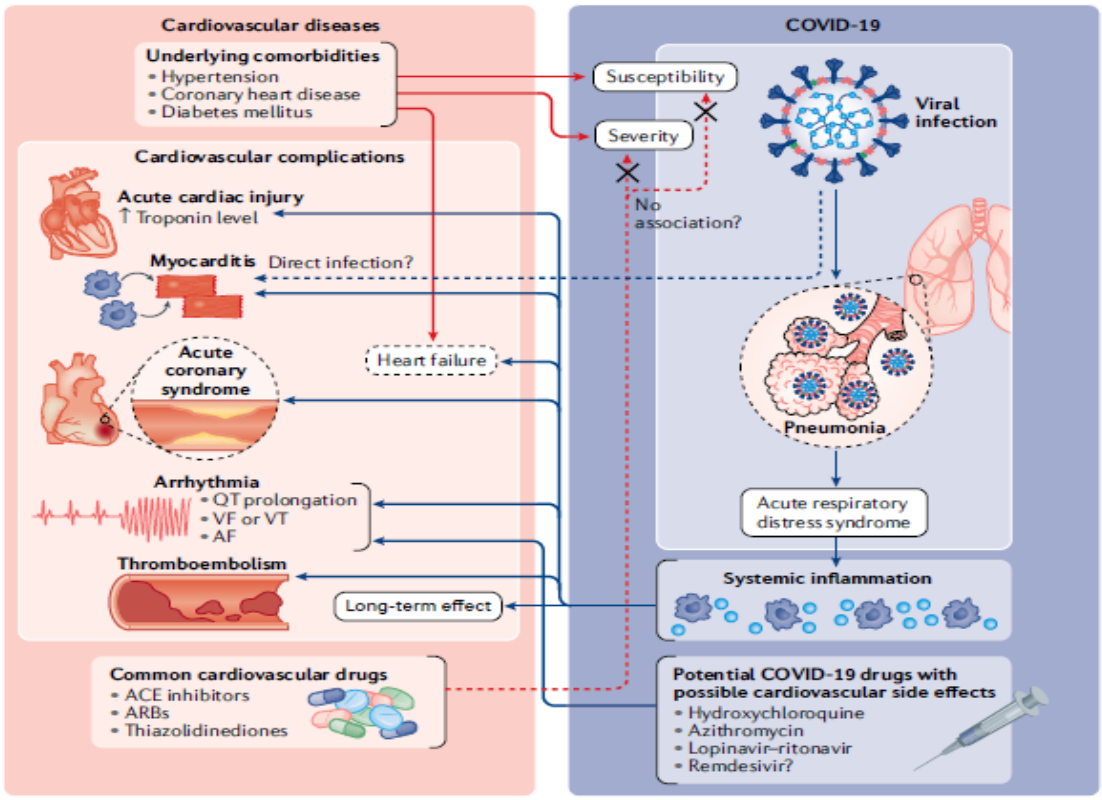

Figure 14 Bidirectional interaction between cardiovascular diseases and COVID-19 [114].

v. Cancer

Cancer is believed to increase the risk of getting infected with severe symptoms of COVID-19 [118] [119] [120]. The reason behind this is the weak immunity due to chemotherapy, the existence of cardiovascular risk factors such as diabetes and hypertension, and the cardiotoxicity of cancer treatment [118]. Furthermore, Liang et al. [120] showed that patients with cancer have a poorer outcome from COVID-19, thus suggesting the potential need to postpone chemotherapy, to wear stronger personal protection provisions, and to observe these patients better, especially if they are older and have at least a single underlying comorbidity.

vi. Chronic Kidney Disease (CKD)

CKD is the comorbidity that is associated with the highest risk for severe COVID-19 [122]. Williamson et al. [123] showed that CKD causes a low-level inflammation and baseline lymphopenia, which are why patients with CKD suffer from severe COVID-19.

vii. Dyslipidemia

Data around dyslipidemia and COVID-19 is a bit controversial though a meta-analysis done by Hariyanto et al. [124] showed that dyslipidemia could increase the risk of getting infected with severe COVID-19. It is believed that the high levels of LDL in patients with dyslipidemia increase the inflammatory gene expression by interacting with macrophages found in atherosclerotic plaques [124]. The high levels of proinflammatory cytokines lead to severe outcomes by causing the occurrence of cytokine storm [125]. Another reason could be the low levels of HDL in patients with dyslipidemia that is inversely related to the C-reactive protein (CRP) levels [126], and poorly regulate the innate immunity that is the defense mechanism of the body against infections [127]. Kim et al. [128] also observed that the buildup of triglycerides and LDL leads to endothelial dysfunction, the latter being important in COVID-19, as it is where ACE2 receptors are expressed [129].

f. Angiotensin Converting Enzyme Inhibitors & ARBs

The RAS is targeted in the treatment of morbidities such as hypertension and heart disease. Drugs include ACE inhibitors (ACE-I) and angiotensin receptor blockers (ARBs) that inhibit the ACE/Ang II/AT1R pathway [130]. ACE inhibitors inhibit the

transformation of angiotensin I into angiotensin II, which will further inhibit the effects of both AT1R and AT2R [131] (**Figure 9**). ACE inhibitors also inhibit the transformation of angiotensin II into angiotensin 1-7; however, the latter would still be formed by endopeptidases which degrade angiotensin I [66].

There are two opposing hypotheses and findings of the effect of ACE inhibitors and ARBs on patients with COVID-19 [132]:

As seen in the right upper panel of **Figure 15**, using RAS inhibitors increases the availability of ACE2, leading to more viral binding compared to the upper left panel of **Figure 15**. This is because inhibiting ACE with ACEIs diminishes the availability of angiotensin II, leading to an increase in the number of free ACE2 receptors available, thus increasing general inflammation.

On the other hand, without RAS inhibitors after SARS-CoV-2 binding, angiotensin II will bind to AT1R, causing inflammation and fibrosis, as seen in the lower left panel of **Figure 15**. However, as seen in the lower right panel of **Figure 15**, using ACE inhibitors decreases the angiotensin II levels and facilitate the production of angiotensin 1-7 and ARBs blocks AT1R and activate Mas receptor, both of which would inhibit inflammation and fibrosis and yield positive effects on the lung.

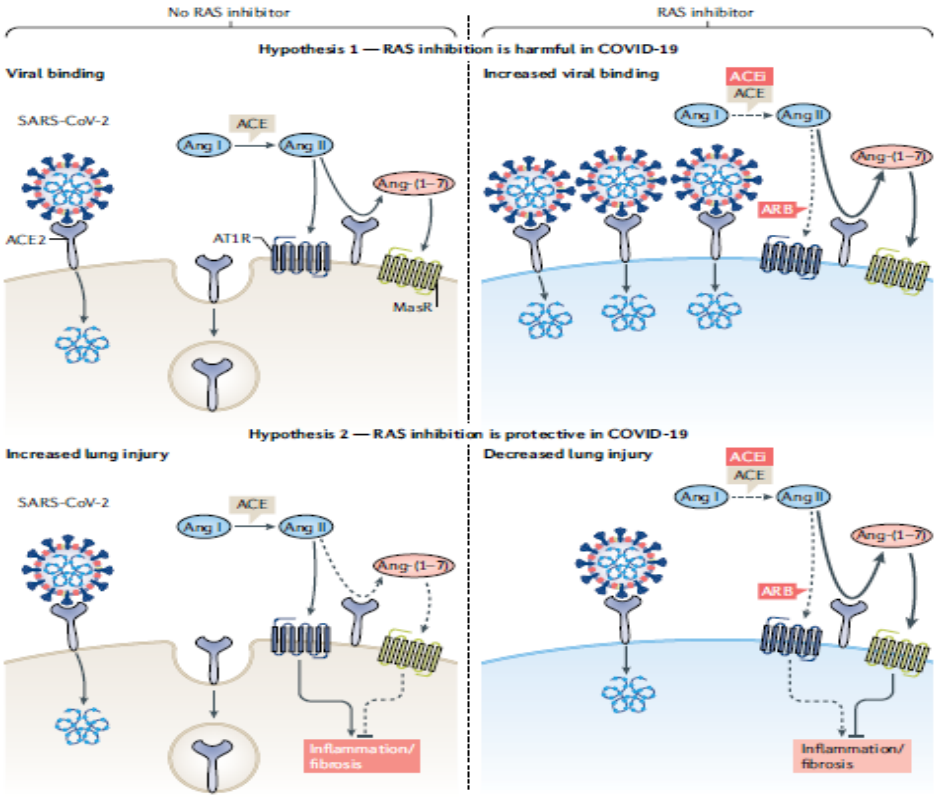

Figure 15 Possible effects of renin-angiotensin system inhibition on COVID-19 [133].

C. Genetics

Despite the absence of obvious risk factors such as age, sex and comorbidities, there may be differences in contracting the COVID-19 disease or its severity, a phenomenon that can be partially explained by genetic susceptibility [134].

To date, there have been a number of studies looking at COVID-19 and host genetics, some of which were based on historic genetic data and others on actual case control studies. Below is a detailed review of the genetic literature for *TMPRSS2*, *ACE2* and *ACE1* genes (**Table 4**).

1. Transmembrane Protease Serine 2

TMPRSS2 is related to COVID-19 [135] since its expression facilitates the entry of SARS-CoV-2 into the host cells through ACE2 [17] [136] [137] [138]. Functional studies have shown that synthetic mutations near the S1-S2 site of the S protein of SARS-CoV-2 increase the viral membrane fusion by several folds [13]. From this data, Bhattacharyya et al. [139] performed an In-Silico analysis to predict the cleavage site by knowing the sequence of the S protein of the COVID-19 virus. They found that there is a geographic spread of different subtypes of SARS-CoV-2, and that the A2a subtype, that causes a non-synonymous Aspartate (D) to Glycine (G) (D614G) mutation in the S protein leading to having an additional cleavage site, has the highest frequency [139]. The spread of A2a subtype started from China in January 2020 which rapidly reached Europe, and by the end of February 2020 the frequency of A2a subtype increased in Europe and North America compared to China and other East Asian countries where the frequency remained low [139]. The A2a subtype alone was, however, not enough to explain the rapid rise in the frequency of SARS-CoV-2 infected individuals in Europe, which led Bhattacharyya et al. [139] to identify 136 eQTLs that are significantly associated with the expression of *TMPRSS2* through GTEx data [140] and for further analysis, they selected non-silent polymorphic variants of *TMPRSS2*. The eQTL rs35074065 was found to have the strongest association whereby the variant allele, which is the deletion of nucleotide C, increases the expression of the TMPRSS2 gene, thus increasing the susceptibility to SARS-CoV-2 infection [139]. DelC was found to be most common in Europeans and less so in East Asians, indicating its potentially protective role against SARS-CoV-2 infection, and concluding that the frequency of A2a subtype is strongly related to the delC allele frequency [139]. Along these same

lines, Russo et al. [141] showed that the allele delC of *TMPRSS2* variant rs35074065 that is common in Europeans and potentially associated with higher COVID-19 related mortality, to significantly increase the expression of *TMPRSS2*. Moreover, the alternative A allele of rs13052975, a common variant in East Asian populations, was found to be protective against severe COVID-19 disease since it decreases the expression of *TMPRSS2* [141].

It has been suggested that the susceptibility and risk of SARS-CoV-2 infection is greatly influenced by genetic variations particularly *ACE2* and *TMPRSS2*, which led Torre-Fuentes et al. [135] to perform a whole-exome sequencing to analyze the frequencies of *ACE2*, *TMPRSS2* and *Furin* genes. The study included a cohort of 120 individuals from Madrid, Spain out of whom 45 were diagnosed with multiple sclerosis (MS) according to the McDonald criteria [142]. The authors found no significant results for *ACE2* and *Furin* genes; however, two synonymous *TMPRSS2* variants rs61735792 and rs6173574 were found to be significantly associated with SARS-CoV-2 infection [135]. Furthermore, Hou et al. [143], by using the quantitative trait loci QTLbase database [144], found an expression quantitative trait loci (eQTL) that is associated with a non-synonymous variant of *TMPRSS2* rs12329760. According to Hou et al. [143] p.Val160Met (rs12329760), which is found in all populations with a high allele frequency (~25%), may explain the distinctive genetic susceptibility to COVID-19.

2. Angiotensin Converting Enzyme 2

The S protein of SARS-CoV-2 is very important in the binding of the virus since it recognizes the peptidase domain of ACE2 receptor [145] [146]. Based on this, Guo et al. [147] analyzed the structural interactions between the ACE2 receptor and the S

protein of SARS-CoV-2. The authors showed that the S protein of SARS binds to ACE2 in both closed and open state. They also stated that the structure of the ACE2 receptor could be deformed by deleterious missense variants [147]. Accordingly, they showed that p.His378Arg (rs142984500) could diminish the catalytic activity of ACE2 receptor and destabilize its structure. They also showed that p.Arg219Cys/His (rs372272603) significantly affects the interactions of the secondary structures [147].

Previous studies have found that rs2285666 variant of the *ACE2* gene is associated with hypertension [148] [149], with the G8790A transition leading to lower *ACE2* activity [150] [151]. Based on these findings, Gomez et al. [154] sequenced intron and exon-flanking sequence of the *ACE2* gene and found rs2285666 to be the only significant variant. Their results were similar to previous studies showing that rs2285666 is associated with hypertension; however, they found no significant difference in patients with mild versus severe COVID-19 disease [152].

It was previously shown by Li et al. [153] that *ACE2* variants can decrease the interaction between the S protein of SARS-CoV and ACE2 receptor. This lead Cao et al. [154] to study the expression level of *ACE2* in different populations as a factor behind the difference in susceptibility and outcome of SARS-CoV-2 infection. Recently, and by performing single-cell RNA sequencing, it was shown that *ACE2* expression is higher in Asian males [155]. In addition, Cao et al. [154] found 7 hotspot variants in the *ACE2* gene that are distributed in different populations. The eQTL rs4646127 variant was found to have a high allele frequency (AF) in the East Asian population (0.994) [154], confirming that the population of East Asia has a higher expression of *ACE2*, hence the difference in susceptibility to SARS-CoV-2 infection when compared to other populations.

To date there have been no data on the association between *ACE2* genetic polymorphisms and severity outcome of COVID-19, until Wooster et al. [156] aimed to study this potential link by genotyping for 61 *ACE2* SNPs that showed to have a significant association with *ACE2* tissue mRNA expression using the GTEx project dataset [157]. 10 SNPs were found to be significantly correlated with *ACE2* expression, out of which 5 SNPs (rs6632680, rs4240157, rs1476524, rs2048683 and rs483065) lead to a significant increase in the tissue expression of *ACE2* which was associated with an increase in the risk of hospitalization in patients with COVID-19 [156]. On the other hand, rs1548474 *ACE2* SNP was found to decrease the tissue expression of *ACE2*, which was associated with lower chances of COVID-19 disease severity and a lesser need for hospitalization [156].

3. Angiotensin Converting Enzyme 1

ACE1 is another important factor besides ACE2 in the renin-angiotensin system, making *ACE1* a candidate gene in the studies of genetic polymorphisms and COVID-19. Most studies evaluated the *ACE1* insertion/deletion (*I/D*) variant that is represented by four identifiers, rs4646994, rs4340, rs1799752 and rs13447447 [75], and was previously shown to be associated with ARDS and poor outcomes [158]. *ACE1 DD* leads to higher activity of the ACE1 enzyme, thus secondarily increasing the levels of Angiotensin II and lowering the expression of *ACE2*. This lowering of *ACE2* could be protective against viral infections, but may lead to higher cardiovascular and lung pathologies due to the higher plasma levels of angiotensin II [152] [159] [160] (**Figure 16**).

As a matter of fact, a study by Adamzik et al. [161] including 84 Caucasian patients with ARDS has shown that patients with the *DD* genotype had a significantly higher risk for COVID-19 related death. In addition, Gomez et al. [152] genotyped for the *I/D* polymorphism of *ACE1* gene in 204 COVID-19 patients (67 severe-ICU and 137 non severe cases) and 536 controls to observe if there is an association with the severity of SARS-CoV-2 infection. They found that the *ACE1 DD* genotype was significantly higher among the severe COVID-19. Moreover, although not significant, *ACE1 DD* genotype was found to be higher in mild, severe and control hypertensive individuals; however, no difference was found between controls and COVID-19 patients [152]. Furthermore, Annunziata et al. [162] aimed to seek whether there is a correlation between *ACE1 I/D* polymorphism and respiratory failure due to COVID-19. Their study included 26 patients with COVID-19, out of whom 24 had severe respiratory failure paO2/FiO2 < 100 mmHg [162]. They found that the critically ill patients with paO2/FiO2 around 75.6+/-11.3 mmHg carried the *ACE1 DD* genotype, while patients with paO2/FiO2 above 200 mmHg presented with *ACE1 II* genotype [162].

The distribution of the *ACE1 I/D* polymorphism was shown to be of major geographical variability [163], which led several investigators to compare its geographical distribution to COVID-19 susceptibility and severity in different populations. For instance, Delanghe et al. [164] showed a significant association between the frequentness of *ACE1 D*-allele in 25 European countries and mortality caused by COVID-19. They also mentioned that both China and Korea have low *D* allele frequencies suggesting that *ACE1 I/D* polymorphism may be the reason for the severe outcome and spread of the SARS-CoV-2 virus in other countries [164].

Moreover, Hatami et al. [7] performed an In Silico analysis to study the relationship between the recovery rate of COVID-19 patients and *ACE1 I/D* polymorphism. They found that, with a higher *I/D* allele frequency ratio, the higher the recovery rate, though they saw no significant change in the death rate [7]. The authors also postulated that since the *I/D* allele frequency ratio is above 1 in East Asian countries (China and Japan), this may be the reason why these populations have a higher recovery rate [7]. Li et al. [165] also supported these results, who showed that America, Africa, Europe and the Arab regions have a higher frequency of allele *D*, while East Asia appeared to have a lower allele *D* frequency. All these data support the hypothesis that *ACE1 I/D* is ethnically and geographically variable, which is associated with the different clinical outcomes of COVID-19, whereby severe disease is experienced by populations who have a higher frequency of *D* allele [166]. Of note, Yamamoto et al. [167] observed that the European and the Middle Eastern populations have a higher probability of getting infected by SARS-CoV-2 compared to other Asian populations. Importantly, their correlation between COVID-19 mortality and *ACE1 II* genotype was weakened when they added data from the Middle East, stating that the Middle East should be considered an important factor for future studies [167]. This is especially the case since, and as per Saab et al. [163], the Middle Eastern population, such as the Lebanese, have a lower frequency of the *ACE1 I* allele when compared to the *D* allele.

In conclusion, the presented data from the currently available literature shows that most of the studied *ACE2* variants lead to a change in the structure of the ACE2 receptor and *ACE2* expression, thus potentially affecting the susceptibility of getting infected by SARS-CoV-2. In comparison, the evaluated *ACE1* variants are related to the

severity of the COVID-19 disease by increasing the activity of the ACE1 enzyme and leading to a reduction in ACE2 levels (See **Table 4**).

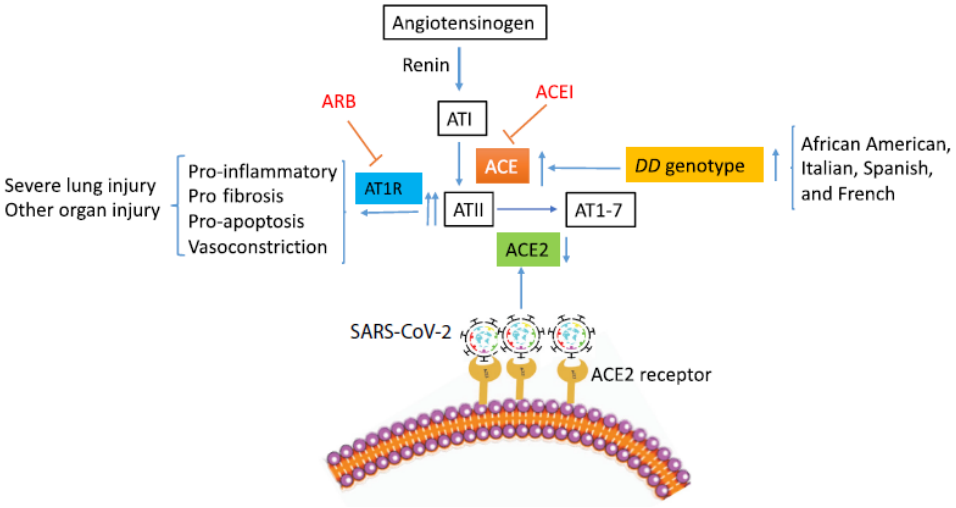

Figure 16 Proposed mechanisms of ACE *DD* genotype in SARS-CoV-2 related severe lung injury and its impact in a high-risk population [166].

Gene	Variant	Type of Study	Outcome	Reference
TMPRSS2	rs35074065	In Silico	Susceptibility	[139]
TMPRSS2	rs61735792 rs61735794	Study	Susceptibility	[135]
TMPRSS2	rs12329760	Big data frequency	Susceptibility	[143]
TMPRSS2	rs35074065	In Silico	Susceptibility	[141]
ACE2	rs372272603 rs142984500	In Silico	Susceptibility	[147]
ACE2 *ACE1*	rs2285666 rs1799752/rs4340	Study	Susceptibility and severity	[152]
ACE2	rs4646127	Big data frequency	Susceptibility	[154]
ACE2	rs4240157 rs6632680 rs4830965 rs1476524 rs2048683 rs1548474	Study	Severity	[156]
ACE1	rs1799752/rs4340	Study	Severity	[161]
ACE1	rs1799752/rs4340	Study	Severity	[162]
ACE1	rs1799752/rs4340	In Silico	Severity	[164]
ACE1	rs1799752/rs4340	In Silico	Severity	[7]

Table 4 Summary of the genetic literature focusing on significant results for the *TMPRSS2*, *ACE2* and *ACE1* genes.

CHAPTER II

AIMS

Individuals infected with the SARS-CoV-2 virus experience different symptoms with varying severity. In addition, not all individuals get infected upon exposure to the virus, hence highlighting inter-individual variability in the incidence and severity of COVID-19. Risk factors such as age, sex and comorbidities play a major role in this variability; however, genetics may also be important in driving the differences in the incidence and severity of the disease.

The SARS-CoV-2 virus enters the host cell by the spike protein (S) binding to ACE2, which is a major factor along with ACE1 in the Renin-Angiotensin System (RAS). Both ACE1 and ACE2 are key factors balancing the RAS, and their genetic polymorphisms might explain the potential link between genetics and the incidence and severity of COVID-19. Few studies show that *ACE1 I/D* polymorphism plays an important role in the severity of the COVID-19 disease. More specifically, some populations were shown to have more of the *D* allele coupled with worse COVID-19 outcomes. Data concerning the risk of contracting the disease is conflicting, and no studies have yet been conducted on the Middle Eastern population such as the Lebanese.

Aim 1: To evaluate the role of *ACE I/D* genetic polymorphism in the risk of contracting the COVID-19 infection

Aim 2: To evaluate the role of *ACE I/D* genetic polymorphism on the outcome of the COVID-19 infection

CHAPTER III

METHODS

A. Human Subjects

The study was approved by the AUB Institutional Review Board (IRB) for participant recruitment and peripheral blood sample collection, and was done in partnership with AUBMC.

1. Recruitment

Recruitment was done at AUB and AUBMC premises. A person is considered to be a potential recruit for the study if he/she presented to AUBMC for a PCR test for COVID-19 with either positive or negative result, or was hospitalized at AUBMC because of COVID infection, or presented to AUBMC for post-COVID persistent symptoms.

After the explanations and acceptance of the individual to participate in the study, an IRB approved informed consent (**Appendix 1**) was signed which allows asking few questions, withdraw or access leftover peripheral blood to be used to perform DNA isolation for ACE genotyping, and access medical records for demographics, comorbidities, medications intake, and COVID-19 disease presentation, management and progression.

Exclusion criteria include children (<18 years old) and individuals with no PCR test done and intubated and cognitively impaired patients who do not have a family or a legal representative.

2. Data Collection

The data collection consisted of filling a questionnaire and accessing the medical records of the participant.

a. Questionnaire and Medical Records

The questionnaire included questions on the date of the PCR test, signs and symptoms upon presentation and history of exposure. The questionnaire is detailed and categorizes the questions for individuals depending upon whether the PCR test was positive or negative and whether a subject with a positive result was hospitalized or not. Missing information in the questionnaire were filled from data from the EPIC electronic medical records especially for hospitalized patients (**Appendix 2**).

B. Blood Collection

Blood withdrawal was performed by the Research Fellow Dr. Halim Saad, who is trained and certified in phlebotomy. Leftover blood was taken for hospitalized patients in order to avoid any extra needle prick.

Peripheral blood (4cc in EDTA containing tubes) was then processed into 300µl aliquots and stored at -80°C until analysis.

C. DNA Isolation

DNA was isolated using FlexiGene® DNA Kit (250) by QIAGEN® (Germany) as per the manufacturer's guidelines.

The isolated DNA was then read using the DS-11 Spectrophotometer (DeNovix®, USA) to quantify and assess the purity of the isolated DNA.

D. Genotyping of the *ACE I/D* polymorphism

Genotyping for the *ACE I/D* polymorphism was performed by Polymerase Chain Reaction (PCR) followed by gel visualization.

1. PCR

PCR was performed using a T100™ Thermal Cycler (BioRad, USA). The primers used in this step are:

Forward: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'

Reverse: 5'-GAT GTG GCC ATC ACA TTC GTC AGAT-3'

The PCR reaction was performed in a final volume of 20µl containing 3µl genomic DNA, 0.5µM of each primer, 1X REDTaq® ReadyMix™ (Sigma-Aldrich, USA) and completed by 3µl of DNase and RNase free water.

The PCR was started on the thermal cycle with an initial denaturation for 95˚C for 2 minutes. Afterward, the DNA was amplified for 30 cycles with denaturation at 94˚C for 30 seconds, annealing at 59˚C for 30 seconds, and extension at 72˚C for 45 seconds. This step was followed by a final extension at 72˚C for 9 minutes.

2. Gel Visualization

The PCR products were then run on a 1% agarose gel stained with 2% ethidium bromide and then visualized by using UV fluorescence Gel Doc™ EZ System (BioRad, USA). A GeneRuler ™ 100bp DNA ladder by Fermentas was used to confirm the length of the fragments. Individuals who were homozygous for the *D* allele were identified by a single 190 bp PCR product, while those who were homozygous for the *I*

allele were identified by the presence of a single 490 bp PCR product. Heterozygous individuals (*ID* genotype) were identified by the presence of two bands of 190 and 490 bp PCR products (**Figure 17**).

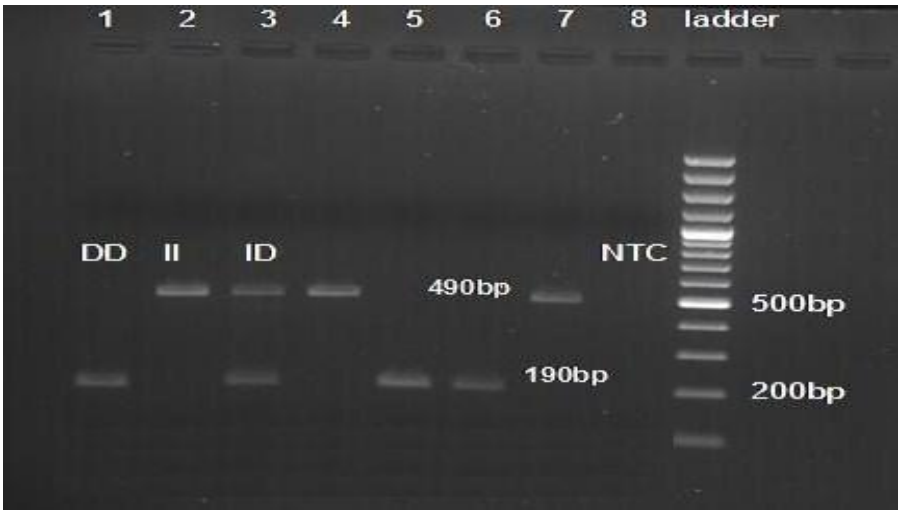

Figure 17 ACE I/D polymorphism detected on 7 DNA samples. Lane 8 is no template control. Lanes 1,5 and 6 are DD. Lane 3 is ID. Lanes 2,4 and 7 are II.

E. Statistical Analysis

Data were entered in excel and then transferred onto the IBM® SPSS® statistical software. A P value of less than 0.05 was considered statistically significant.

Association analyses were done using Fisher's Exact test for categorical variables and independent sample t-test for continuous variables. Binary logistic regression was used for the multivariate analysis after adjusting for statistically significant covariates at the univariate level. Results are presented as number (percentage), mean ± standard deviation (SD), and Odds Ratios (OR) with 95% Confidence Intervals (CI) as applicable.

For aim 1, cases were compared to controls, and for aim 2 we did three types of analyses: mild + moderate vs. severe disease, hospitalized vs. non-hospitalized, and hypoxic vs non-hypoxic. Disease severity was categorized based on the WHO criteria, and hypoxia was defined as an oxygen saturation level below 94% [63]. In addition, and knowing that ACE *D* allele is known to be associated with some of the comorbidities such as hypertension and cardiac diseases, an additional exploratory analysis was performed to look at such an association in patients. This will be followed by a multivariate or stratification analyses once recruitment is closed.

Baseline characteristics included in the analysis were age, body mass index (BMI), sex, blood group (categorized as being A+ or not), smoking (categorized as never, former and current), cancer, intake of ACEI or ARBs, and comorbidities. The comorbidities included were diabetes, hypertension (HTN), CVD, CKD, lung diseases (COPD and asthma), cerebral vascular disease and coagulation disorders. Dyslipidemia was not initially included because of the controversial results and because most of the dyslipidemic patients already had additional co-morbidities. This will be integrated at the final analysis once recruitment is closed.

The association with the ACE genotype was performed as three categories (*II* vs. *DI* vs. *DD*) and as 2 different combinations (*II* vs *DI*+*DD* and *DD* vs *II*+*DI*).

CHAPTER IV

RESULTS

As of June 2021, more than 350 participants have been recruited into the study, but the below analysis is on 266 subjects for whom data is complete. This cohort entails 124 (46.62%) controls (PCR negative) and 142 (53.38%) cases (PCR positive). Recruitment will remain open until end of July 2021. The *D* allele was more common with the control subjects being 46.8% *DD*, 45.2% *ID*, and 8.1% *II*, with the *D* allele frequency being: 69%.

A. Aim 1: Risk of contracting the viral disease

1. Baseline Characteristics

When comparing cases to controls, and as expected, the cases were significantly older (mean ± SD age in years of 43.04 ± 16.56 vs 38.14 ± 12.00 respectively), and they had significantly higher BMI (kg/m^2): 27.79 ± 5.40 vs 26.04 ± 4.20 respectively. The cases were also more males than females (56.3% vs 46.8% respectively) although this was not statistically significant. In addition, and as expected, comorbidities were significantly higher among the cases, and the latter were significantly more former smokers (**Table 5**).

2. Association with the ACE genotype

As seen in **Table 5**, there were no statistically significant associations of the ACE genotype with the risk of contracting the disease although it is noted that more of the *II* were cases than controls (15.5% vs. 8.1% respectively). As a matter of fact, when

doing a multivariate logistic regression adjusting for age, BMI, smoking and comorbidity covariates, it turned out that participants with the *II* genotype were at a higher risk of contracting the disease when compared to those carrying the *D* allele (*DI+DD*) with an OR of 2.373 (P=0.037) (**Table 6**).

			Controls N=124	**Cases N=142**	**P-Value**
Age (Years)		Mean ± SD	38.14 ± 12.00	43.04 ± 16.56	**0.007**
BMI (kg/m^2)		Mean ± SD	26.04 ± 4.20	27.79 ± 5.40	**0.004**
Sex	**Female**	N (%)	66 (53.2)	62 (43.7)	0.076
	Male	N (%)	58 (46.8)	80 (56.3)	
Blood Group A+	**Yes**	N (%)	66 (53.2)	79 (55.6)	0.394
	No	N (%)	58 (46.8)	63 (44.4)	
Smoking	**Current**	N (%)	43 (34.7)	44 (31.0)	**0.042**
	Former	N (%)	5 (4.0)	18 (12.7)	
	Never	N (%)	76 (61.3)	80 (56.3)	
Comorbidity[1]	**Yes[1]**	N (%)	25 (20.2)	47 (33.1)	**0.012**
	No	N (%)	99 (79.8)	95 (66.9)	
Cancer	**Yes**	N (%)	3 (2.4)	8 (5.6)	0.158
	No	N (%)	121 (97.6)	134 (94.4)	
ACEI or ARBs	**Yes**	N (%)	9 (7.3)	16 (11.3)	0.182
	No	N (%)	115 (92.7)	126 (88.7)	
***ACE* Genotype**	***II***	N (%)	10 (8.1)	22 (15.5)	0.155
	DI	N (%)	56 (45.2)	63 (44.4)	
	DD	N (%)	58 (46.8)	57 (40.1)	
II* vs *DI+DD	***II***	N (%)	10 (8.1)	22 (15.5)	0.46
	DI+DD	N (%)	114 (91.9)	120 (84.5)	
DD* vs *II+DI	***DD***	N (%)	58 (53.2)	85 (59.9)	0.167
	II+DI	N (%)	66 (46.8)	57 (40.1)	

Table 5 Comparison of baseline characteristics & ACE1 genotype between controls and cases.

[1] Yes having any of the following comorbidities: diabetes, hypertension (HTN), cardiovascular diseases (CVD), chronic kidney diseases (CKD), lung diseases (COPD and asthma), cerebral vascular disease and coagulation disorders.

		OR	95% CI	P-Value
II* vs *DI+DD	Cases .vs Controls	**2.373**	**1.052 – 5.356**	**0.037**
DD* vs *II+DI	Cases vs Controls	0.832	0.501 – 1.380	0.476

Table 6 Association of ACE1 genotype with risk of contracting COVID-19 disease. Odds ratios and 95% confidence interval were generated using multivariate binary logistic regression.

B. Aim 2: Viral disease outcome

1. Univariate Analyses

a. Severity

Out of the 142 PCR positive cases, 6 (4.2%) were asymptomatic and are hence excluded from this analysis. Among the 136 symptomatic cases, 88 (64.70%) had mild, 16 (11.76%) had moderate and 32 (22.5%) had severe disease. The mean ± SD duration of symptoms was 8.92 ± 7.33 days with that of mild, moderate and severe disease being 10.38 ± 6.82, 6.33 ± 3.51, and 10.00 ± 0.00 days, respectively. When comparing the combination of mild and moderate to severe cases (**Table 7**), and as expected, the severe cases were significantly older (mean ± SD age in years of 59.91 ± 16.26 vs. 38.32 ± 13.35 respectively) and had a significantly higher BMI (kg/m2): 30.403 ± 6.58 vs 27.098 ± 4.86 respectively). There were more males among the severe cases (71.9% vs 53.8% respectively) which was almost significant (P=0.053). In addition, those who had severe diseases were significantly more former smokers and had significantly more comorbidities. The latter were also taking more ACEI or ARBs as a treatment for their diseases such as CKD and HTN.

			Mild+Moderate[1] N=104	Severe[2] N=32	P-Value
Age (Years)		Mean ± SD	38.32 ± 13.35	59.91 ± 16.26	< **0.001**
BMI (kg/m^2)		Mean ± SD	27.098 ± 4.86	30.403 ± 6.58	**0.003**
Sex	**Female**	N (%)	48 (46.2)	9 (28.1)	0.053
	Male	N (%)	56 (53.8)	23 (71.9)	
Blood Group A+	**Yes**	N (%)	58 (55.8)	17 (53.1)	0.475
	No	N (%)	46 (44.2)	15 (46.9)	
Smoking	**Current**	N (%)	37 (35.6)	5 (15.6)	**0.002**
	Former	N (%)	8 (7.7)	10 (31.3)	
	Never	N (%)	59 (56.7)	17 (53.1)	
Comorbidity[3]	**Yes**	N (%)	25 (24.0)	22 (68.8)	< **0.001**
	No	N (%)	79 (76.0)	10 (31.3)	
Cancer	**Yes**	N (%)	4 (3.8)	4 (12.5)	0.088
	No	N (%)	100 (96.2)	28 (87.5)	
ACEI or ARBs	**Yes**	N (%)	8 (7.7)	8 (25.0)	**0.013**
	No	N (%)	96 (92.3)	24 (75.0)	
***ACE* Genotype**	***II***	N (%)	19 (18.3)	3 (9.4)	0.342
	DI	N (%)	47 (45.2)	13 (40.6)	
	DD	N (%)	38 (36.5)	16 (50.0)	
II* vs *DI+DD	***II***	N (%)	19 (18.3)	3 (9.4)	0.180
	DI+DD	N (%)	85 (81.7)	29 (90.6)	
DD* vs *II+DI	***DD***	N (%)	38 (36.5)	16 (50)	0.125
	II+DI	N (%)	66 (63.5)	16 (50)	

Table 7 Comparison of baseline characteristics & ACE1 genotype between mild+moderate and severe cases.

[1] Mild means that the person can experience general initial symptoms such as fever, chills, cough, nasal congestion, headache and sore throat. However, they might also experience gastrointestinal symptoms such as nausea, vomiting and diarrhea. There could be loss of smell (anosmia) or loss of taste (ageusia). The disease is categorized moderate when the subject is hospitalized with or without oxygen therapy according to the WHO clinical progression scale [63]. We categorized the few subjects who received oxygen therapy at home as moderate.

[2] According to the WHO clinical progression scale having severe symptoms means that when the subject is hospitalized, they require oxygen by non-invasive ventilation (NIV) or high flow, intubation, mechanical ventilation, vasopressors and dialysis [63].

[3] Yes having any of the following comorbidities: diabetes, hypertension (HTN), cardiovascular diseases (CVD), chronic kidney diseases (CKD), lung diseases (COPD and asthma), cerebral vascular disease and coagulation disorders.

b. Hospitalization

Among the cases, 46 (32.4%) subjects were hospitalized with a mean ± SD duration of stay of 3.49 ± 6.758 days, while 96 (67.6%) subjects were not hospitalized. When comparing the baseline characteristics between hospitalized and non-hospitalized subjects (**Table 8**), age (years) (mean ± SD of 56.93 ± 16.90 vs. 36.39 ± 11.55) and BMI (kg/m2) (mean ± SD of 29.426 ± 6.06 vs. 27.016 ± 4.89) were significantly higher in the hospitalized compared to non-hospitalized patients respectively. The hospitalized subjects were significantly more males 33 (71.7%) vs. 47 (49.0%) respectively. As expected, former smoking, comorbidities, intake of ACEI/ARBs and cancer were also significantly higher in the hospitalized subjects.

			Not Hospitalized	Hospitalized	P-Value
Age (Years)		Mean ± SD	36.39 ± 11.55	56.93 ± 16.90	< **0.001**
BMI (kg/m^2)		Mean ± SD	27.016 ± 4.89	29.426 ± 6.06	**0.012**
Sex	**Female**	N (%)	49 (51.0)	13 (28.3)	**0.008**
	Male	N (%)	47 (49.0)	33 (71.7)	
Blood Group A+	**Yes**	N (%)	55 (57.3)	24 (52.2)	0.346
	No	N (%)	41 (42.7)	22 (47.8)	
Smoking	**Current**	N (%)	37 (38.5)	7 (15.2)	< **0.001**
	Former	N (%)	5 (5.2)	13 (28.3)	
	Never	N (%)	54 (56.3)	26 (56.5)	
Comorbidity[1]	**Yes**	N (%)	17 (17.7)	30 (65.2)	< **0.001**
	No	N (%)	79 (82.3)	16 (34.8)	
Cancer	**Yes**	N (%)	1 (1.0)	7 (15.2)	**0.002**
	No	N (%)	95 (99.0)	39 (84.8)	
ACEI or ARBs	**Yes**	N (%)	6 (6.3)	10 (21.7)	**0.009**
	No	N (%)	90 (93.8)	36 (78.3)	
***ACE* Genotype**	***II***	N (%)	17 (17.7)	5 (10.9)	0.563
	DI	N (%)	42 (43.8)	21 (45.7)	
	DD	N (%)	37 (38.5)	20 (43.5)	
II* vs *DI*+*DD	***II***	N (%)	17 (17.7)	5 (10.9)	0.213
	DI*+*DD	N (%)	79 (82.3)	41 (89.1)	
DD* vs *II*+*DI	***DD***	N (%)	37 (38.5)	20 (43.5)	0.351
	II*+*DI	N (%)	59 (61.5)	26 (56.5)	

Table 8 Comparison of baseline characteristics & ACE1 genotype between hospitalized and non-hospitalized cases.

[1] Yes having any of the following comorbidities: diabetes, hypertension (HTN), cardiovascular diseases (CVD), chronic kidney diseases (CKD), lung diseases (COPD and asthma), cerebral vascular disease and coagulation disorders.

c. Hypoxia

Out of the 46 hospitalized subjects, 33 (71.74%) were hypoxic and 13 (28.26%) were not hypoxic. When comparing baseline characteristics between hypoxic and non-hypoxic subjects (**Table 9**), hypoxic subjects were significantly older (mean ± SD of 60.09 ± 16.04 vs. 48.92 ± 16.97 years respectively) and had significantly higher BMI (kg/m2) (mean ± SD of 30.545 ± 6.53 vs 26.585 ± 3.42 respectively).

			Not Hypoxic	Hypoxic	P-Value
Age (Years)		Mean ± SD	48.92 ± 16.97	60.09 ± 16.04	**0.042**
BMI (kg/m^2)		Mean ± SD	26.585 ± 3.42	30.545 ± 6.53	**0.045**
Sex	**Female**	N (%)	4 (30.8)	9 (27.3)	0.541
	Male	N (%)	9 (69.2)	24 (72.7)	
Blood Group A+	**Yes**	N (%)	7 (53.8)	17 (51.5)	0.574
	No	N (%)	6 (46.2)	16 (48.5)	
Smoking	**Current**	N (%)	2 (15.4)	5 (15.2)	0.903
	Former	N (%)	3 (23.1)	10 (30.3)	
	Never	N (%)	8 (61.5)	18 (54.5)	
Comorbidity[1]	**Yes**	N (%)	7 (53.8)	23 (69.7)	0.248
	No	N (%)	6 (46.2)	10 (30.3)	
Cancer	**Yes**	N (%)	3 (23.1)	4 (12.1)	0.305
	No	N (%)	10 (76.9)	29 (87.9)	
ACEI or ARBs	**Yes**	N (%)	1 (7.7)	9 (27.3)	0.146
	No	N (%)	12 (92.3)	24 (72.7)	
***ACE* Genotype**	***II***	N (%)	2 (15.4)	3 (9.1)	0.570
	DI	N (%)	7 (53.8)	14 (42.4)	
	DD	N (%)	4 (30.8)	16 (48.5)	
II* vs *DI+DD	***II***	N (%)	2 (15.4)	3 (9.1)	0.439
	DI+DD	N (%)	11 (84.6)	30 (90.9)	
DD* vs *II+DI	***DD***	N (%)	4 (30.8)	16 (48.5)	0.225
	II+DI	N (%)	9 (69.2)	17 (51.5)	

Table 9 Comparison of baseline characteristics & ACE1 genotype between hypoxic and non-hypoxic cases.

[1] Yes having any of the following comorbidities: diabetes, hypertension (HTN), cardiovascular diseases (CVD), chronic kidney diseases (CKD), lung diseases (COPD and asthma), cerebral vascular disease and coagulation disorders.

2. Multivariate Analyses

a. Severity

As seen in **Table 7**, there were no significant associations of the *ACE* genotype with disease severity, although *DD* [16 (50.0%)] was seen to be higher than *DI* [13 (40.6%)] and *II* [3 (9.4%)] in patients who had severe disease. As a matter of fact, when doing a multivariate logistic regression analysis adjusting for age, BMI, smoking, comorbidity and ACEI/ARB's covariates, it turned out that participants with the *DD* genotype were at a higher risk of having the severe disease when compared to those carrying the *I* allele (*II+DI*) with an OR of 7.173 (P=0.003) (**Table 10** and **Figure 18**).

b. Hospitalization

As seen in **Table 8**, there were no significant associations of the *ACE* genotype with hospitalization, although *DD* was higher in the hospitalized group compared to the non-hospitalized (43.5% vs 38.5% respectively). As a matter of fact, after performing the multivariate logistic regression analysis and adjusting for age, BMI, smoking, comorbidity, ACEI/ARBs and cancer covariates, it turned out that participants with the *DD* genotype were at a higher risk of being hospitalized when compared to those carrying the *I* allele (*II+DI)* with an OR of 3.398 (P=0.027) (**Table 10** and **Figure 18**).

c. Hypoxia

As seen in **Table 9**, there were no significant associations of the *ACE* genotype with hypoxia among hospitalized patients though *DD* was higher, but not significantly, in the hypoxic compared to the non-hypoxic patients (48.5% vs 30.8% respectively). Nevertheless, performing the multivariate logistic regression analysis by adjusting for

age and BMI covariates showed that hospitalized participants with the *DD* genotype were at a higher risk of having hypoxia when compared to those carrying the *I* allele (*II+DI*) with an OR of 4.735 (P=0.088) (**Table 10** and **Figure 18**).

		OR	**95% CI**	**P-Value**
II* vs *DI+DD	(Mild+Moderate) vs Severe	0.629	0.125 – 26.478	0.575
	Hospitalized vs Non-hospitalized	0.545	0.124 – 2.392	0.421
	Hypoxic vs Non-hypoxic	0.603	0.066 – 5.492	0.653
DD* vs *II+DI	(Mild+Moderate) vs Severe	**7.173**	**1.943 – 26.478**	**0.003**
	Hospitalized vs Non-hospitalized	**3.398**	**1.151 – 10.032**	**0.027**
	Hypoxic vs Non-hypoxic	**4.735**	**0.794 – 28.236**	**0.088**

Table 10 Association of *ACE1* genotype with risk of COVID-19 severity. Odds ratios and 95% confidence interval were generated using multivariate binary logistic regression.

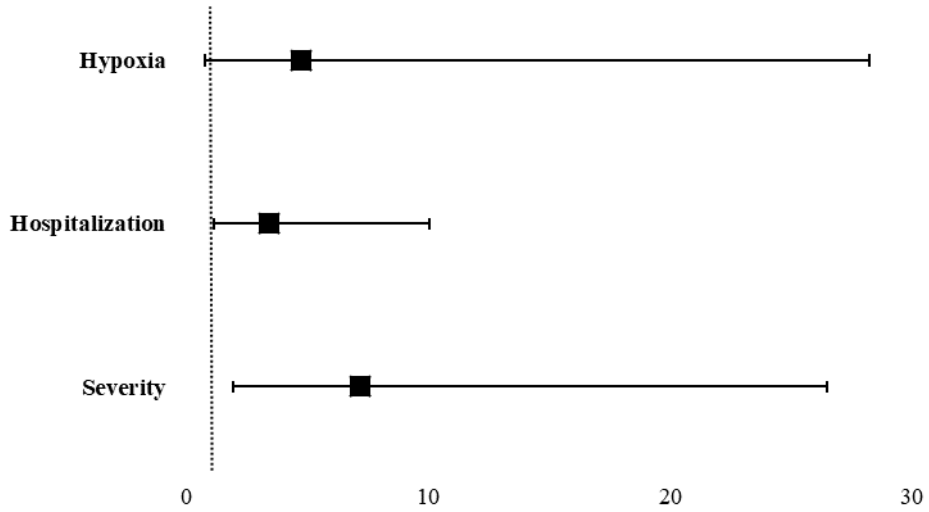

Figure 18 Forest plot showing Odds Ratios and 95% Confidence Intervals of the association of the ***DD*** genotype with disease severity, hospitalization, and hypoxia compared to *I* allele carriers.

C. Association of the *ACE* genotype with comorbidities

As noted in **Tables 7**, **8** and **9**, the *DD* genotype is more common among subjects with severe COVID-19 and those who were hospitalized or had hypoxia. However, this could also be due to the underlying comorbidities that these subjects have. Nevertheless, and as seen in **Table 11**, there were no significant associations between the *ACE* genotype and the mentioned comorbidities, meaning that the *ACE* genotype is the reason behind the severity of the COVID-19 outcome and not the underlying comorbidities.

			II+DI	***DD***	**P-value**
Hypertension	**Yes**	N (%)	21 (72.4)	8 (27.6)	0.09
	No	N (%)	64 (56.6)	49 (43.4)	
Diabetes	**Yes**	N (%)	14 (73.7)	5 (26.3)	0.142
	No	N (%)	71 (57.7)	52 (42.3)	
Heart Disease	**Yes**	N (%)	9 (75.0)	3 (25.0)	0.211
	No	N (%)	76 (58.5)	54 (41.5)	
Kidney Disease	**Yes**	N (%)	5 (83.3)	1 (16.7)	0.225
	No	N (%)	80 (58.8)	56 (41.2)	
Lung Disease	**Yes**	N (%)	4 (40.0)	6 (60.0)	0.160
	No	N (%)	81 (61.4)	51 (38.6)	
Cerebral Vascular Disease	**Yes**	N (%)	2 (100.0)	0 (0.0)	0.357
	No	N (%)	83 (59.3)	57 (40.7)	
Coagulation Disorders	**Yes**	N (%)	2 (100.0)	0 (0.0)	0.357
	No	N (%)	83 (59.3)	57 (40.7)	

Table 11 Association of the *ACE* genotype with comorbidities.

CHAPTER V

DISCUSSION

Ever since the outbreak, it has been realized that the SARS-CoV-2 virus hits every individual differently with varying symptoms and severity. There are many articles discussing the factors that are considered to be risk factors for both symptoms and severity of the COVID-19 disease, but with only few related to genetics. This study shows that almost all previously reported factors and comorbidities also predict disease susceptibility and severity in the Lebanese. We have also shown a positive correlation between the *ACE1 I*-allele and the risk of contracting the COVID-19 disease, and between *ACE1 D*-allele and worse outcome of the COVID-19 infection.

Age is considered an important risk factor since the virus affects the older generation far worse than the younger. ACE2 receptor, being the key factor in the entry of the virus, is higher in number in well-differentiated ciliated epithelial cells found in adults [10]. Moreover, the immunity of an older individual is weaker than the immunity of children due to immunosenescence and the presence of central memory T cells rather than naïve T cells [10]. Our results agree with the literature since the mean ± SD of age (in years) is significantly higher in the cases when compared to the controls (**Table 5**), and it is significantly higher with disease severity (**Tables 7**, **8**, and **9**).

ACE2 being an X-linked gene is considered a disadvantage in males in any polymorphisms or mutations [75]. Moreover, testosterone suppresses the immune system in males, which affects the T cell responses [76]. These findings are compatible with our results that show that 71.7% of the hospitalized subjects were males (**Table 8**).

A study has shown that the frequency of individuals with blood group A was significantly higher in the COVID-19 infected patients' group while blood group O was

significantly lower [81]. However, it was also mentioned that more of the individuals with blood group A had underlying comorbidities [81], which could be the reason behind the significance seen in the infected patients group. In our study, blood group did not show any significant difference with either risk or severity.

Smoking is, by far, the most common risk factor for many diseases. That is why smoking is expected to further complicate the symptoms of COVID-19. Smoking is shown to increase the gene expression of *ACE2* in the lungs [89]. Moreover, nicotine upregulates the activity of renin and ACE thus activating ACE/Angiotensin II/AT1R pathway and decreases the activity of AT2R by downregulating the activity of ACE2 [93]. However, a preliminary meta-analysis on five studies in China has shown that active smoking is not significantly related to the severity of COVID-19 [168]. A systematic review has also shown that current smokers had lower odds ratio for severe outcome when compared to former smokers [169]. This review is in agreement with our results, where former smokers (**Table 5**, **7**, and **8**) were significantly higher in cases when compared to controls. Former smokers also had more severe disease outcome.

Adipose tissues are known to express ACE2 receptors as much as the pulmonary tissues [170]. Accordingly, obese individuals have higher levels of circulating ACE2 with secondarily higher disease susceptibility and adverse outcome [102]. In our study, the mean ± SD of BMI (kg/m2) was significantly higher in the cases and specifically in subjects who were hospitalized and developed hypoxia (**Table 5**, **7**, **8**, and **9**).

To date, it is still unclear whether ACEI and/or ARBs should be kept in patients who contract COVID-19. There are currently 2 contradicting hypotheses in the literature that RAS inhibition could be both harmful and protective [132]. In our study, ACEI/ARBs were significantly more frequently taken in the severe disease group

compared to the mild and moderate (**Table 7**) and in the hospitalized patients compared to the non-hospitalized subjects (**Table 8**). However, it appears that these results are related to the fact that these subjects have comorbidities that necessitate ACEI/ARB's treatment. As a matter of fact, people with underlying comorbidities such as diabetes, hypertension (HTN), cardiovascular diseases (CVD), chronic kidney diseases (CKD), lung diseases (COPD and asthma), cerebral vascular disease and coagulation disorders are at a higher risk of COVID-19 severity sometimes leading to ARDS [95] [171]. Our results clearly show that having comorbidities is significantly higher in the cases when compared to the controls (P=0.012) (**Table 5**). It is also significantly higher in the severe cases (P<0.001) and those hospitalized (P<0.001) (**Tables 7** and **8**).

Concerning *ACE1* genetic factors, it was previously shown that Middle Easterns have a higher frequency of the *ACE1 D*-allele when compared to the I-allele [163]. Moreover, we have previously shown that the *D*-allele is common in the Lebanese population (35.2% *DD*, 51.9% *ID*, and 12.9% *II*) [172]. Similarly in the current study, the *D* allele was more common with the control subjects being 46.8% *DD*, 45.2% *ID*, and 8.1% *II*, with the *D* allele frequency being: 69%.

In relation to disease susceptibility, available data, both of which are so far in-silico, are contradictory. On one hand, Yamamoto et al. [167] showed that countries with higher frequency of the *ACE1 I*-allele had less susceptibility to COVID-19. On the other hand, Delanghe et al [173] showed that a high frequency of *ACE1 I*-allele increases the prevalence of COVID-19 cases. Nevertheless, when Yamamoto et al [167] specifically looked at Middle Eastern populations, they found that their data actually weaken the association with the *D* allele, hence the need for further investigations. To our knowledge, we are the first to evaluate such an association in patients. We

confirmed Delanghe et al.'s simulations by showing that the frequency of *II* was significantly highest (P=0.037) in cases when compared to controls stating that individuals with the *II* genotype have a higher risk (OR=2.373) for contracting the COVID-19 disease.

As for disease severity, *ACE1 DD* genotype leads to higher activity of ACE1 enzyme thus lowering ACE2 causing an increase in the amount of angiotensin II left active. Although lower levels of ACE2 could mean that there is less chance for SARS-CoV-2 to bind and enter the host cell, high levels of angiotensin II would act through AT1R and further cause cardiovascular and lung pathologies [152]. For instance, Gomez et al. [152] found that *ACE1 DD* genotype was more frequent in severe COVID-19 cases, suggesting that there is an association between *ACE1 DD* genotype and the severity of COVID-19. Furthermore, *ACE1 DD* genotype has been correlated with respiratory failure [162] and increased death rate [161] in patients infected with COVID-19. In addition, an in-silico analysis showed that there is a link between *ACE1 I/D* polymorphism and the recovery rate of COVID-19, whereby more of the I allele was shown to be correlated with higher frequency ratio of the *I/D* allele [7]. Our results are in agreement with the literature, whereby *DD* vs *II+DI* (see **Table 10**) was significantly higher in cases with severe disease when compared to mild and moderate disease (P=0.003), hospitalized compared to non-hospitalized cases (P=0.027), and hypoxic compared to non-hypoxic cases (P=0.088). This means that individuals with *DD* genotype have a higher probability to experience severe COVID-19 symptoms (OR=7.173), to be hospitalized (OR=3.398), and/or to be hypoxic (OR=4.735).

CHAPTER VI

LIMITATIONS

This study suffers from several limitations. First, our sample size is small and limited to a single country. Recruitment was done from central Beirut, at AUB only. Second, we only looked at *ACE1* insertion/deletion (*I/D*) variant and did not look at other possible SNPs in *ACE1*. Moreover, it is also important to look at *ACE2* and *TMPRSS2* genetic polymorphisms, as these two genes are important factors in the entry of SARS-CoV-2. Also, there is still no explanation to why former smokers are more likely to have adverse outcomes.

CHAPTER VII

CONCLUSION

Our results show a positive correlation between the *ACE1 I*-allele and the risk of contracting the COVID-19 disease, and between *ACE1 D*-allele and worse outcome of the COVID-19 infection. These results suggest that genotyping for *ACE1 I/D* in parallel to COVID-19 testing could be used to elicit the disease risk and severity for better prognosis and management. We believe that the whole world needs a COVID-19 genetics initiative for all ethnicities and populations. This would help compare the databases among each population to hopefully better explain why Lebanon has had a high number of COVID-19 cases but with a relatively low death rate.

CHAPTER VIII

FUTURE PERSPECTIVES

In the future, we plan:

- To continue recruitment to potentially reach significance with the hypoxia analysis, to analyze severity as mild vs moderate vs severe, and to decrease the confidence interval range.
- To include medications (chloroquine, aspirin, steroids…) in our analysis.
- To look at other candidate genes and if possible, perform whole exome sequencing for significant genetic polymorphisms.
- To evaluate the potential interaction between *ACE I/D* genetic polymorphism and ACEIs and ARBs in COVID-19 infections.
- To associate the cycle threshold (Ct) values of the COVID-19 PCR tests with disease severity
- To look at thrombosis and inflammation outcome within cases

APPENDIX A

Consent to participate in a research study

The Role of Angiotensin Converting Enzyme Insertion/Deletion Genetic Polymorphism in the Risk, Severity and Prognosis of COVID-19 infection

Principle Investigator: Nathalie K. Zgheib
Co-Investigators: Rami Mahfouz, Imad Bou Akl, Carine Sakr

Address: **American University of Beirut Medical Center (AUBMC)**
Beirut, Lebanon
Phone: **(01) 350 000**

You are being asked to participate in a research study conducted at the American University of Beirut. This study has been approved by the Institutional Review Board at AUB for compliance with ethical standards. Please take time to read the following information carefully before you decide whether you want to take part in this study or not. Feel free to ask your doctor if you need more information or clarification about what is stated in this form and the study as a whole.

1) **What is this study about?**

The COVID-19 virus, also known as CORONA, is a new virus that has become pandemic in early 2020, and is still a concern in Lebanon and worldwide. As you may know, for some reason some people do net get infected with the COVID-19 virus even if exposed to infected people. Also, some of the infected people develop a very severe disease while others don't. Therefore the question is: is there a genetic reason behind these inter-individual differences?

The objectives of this genetic study are to determine whether the ACE I/D genetic polymorphism can be used as a marker for risk, severity and prognosis of COVID-19 infections. It is plausible that subjects carrying the ACE I allele are at a higher risk of infection and worse disease presentation and prognosis.

Any subject who presents (or has previously presented) to AUBMC for COVID-19 testing is a potential recruit to the study irrespective of the test result and whether the subject was seen as in- or out-patient. We aim to recruit about 500 subjects. Participants will be seen only once (1 visit) for one blood draw only, and the expected duration of participation is about 1 hour.

After signing this informed consent, the following will occur: We will withdraw peripheral blood for DNA isolation and ACE genotyping; access medical records for demographics, comorbidities, medications intake, COVID-19 disease presentation, and management and progression; and ask few follow up questions on how you have been doing after you came for testing. These questions will take no more than 5 minutes of your time. The genetic test will be performed by a classic PCR. If you are recruited while hospitalized for COVID-19 infection, then you will be asked to sign a copy of the informed consent document, take a picture of the signed form, and send it to the Research Fellow via WhatsApp. There will be no extra needle prick as we will take left over extra blood.

The total volume of blood required is 4 cc which is less than 1 tablespoon. Blood and DNA samples will be stored for genetic testing. Storage of these samples will be in Dr. Zgheib's laboratory, for an indefinite period of time.

2) Any risks as a result of participating in the study?

Although any study may be associated with any unforeseeable risk, this study has minimal risk except for the potential risk of genetic testing as it can reveal information about other family members concerning potential link between ACE genotype and COVID-19 risk in addition to the person who is tested.

None of the data collection measures bare any long term hazards, and the blood withdrawn will be done under sterile hygienic conditions. Possible side effects include mild pain, bleeding, bruising at the site of the needle insertion. Fainting or light-headedness can sometimes occur, but usually last only a few minutes. Note that whether you elect to participate in this study or not does not affect your medical management at all.

You are free to withdraw this consent and discontinue participation in this project at any time. If you elect to do so, you will be asked to send us a signed request after which your blood samples will be destroyed, and your data will be deleted from the study under the supervision of the principal investigator.

AUBMC will cover the cost of treating, on its premises, medical adverse events resulting directly from the medication and/or medical procedures of this research study. Otherwise, it will not cover for the costs of medical care for any medical condition or issue.

3) Any benefits as a result of participating in the study?

It is possible that the information that comes out of the results of the study will contribute to the medical general knowledge on covid-19 infections. It will shed light on whether ACE genotype is a risk factor for COVID-19 infection, and whether it is also a marker for more severe disease and worse prognosis. Should this be the case, then we may suggest genotyping for ACE I/D in parallel to COVID-19 testing to guide healthcare practitioners on stratifying disease risk and severity, and manage and prognosticate accordingly.

You will not be reimbursed for participating in the study. You will however be reimbursed with 20,000 LL for any parking or transportation cost. There will be no additional costs.
The investigators may choose to end your participation. They will convey to you any significant new findings.

4) Confidentiality

Your clinical data, blood and genetic material will be securely stored in Dr. Zgheib's lab at AUB indefinitely. Your samples will however be destroyed if you elect to withdraw your consent for the study. All data collected will be stored in a coded manner with the PIs and the CITI certified Research Assistant. These measures will all be conducted ensuring there is no breach of participants' privacy. You may ask that we provide you with the genetic results and explain their significance to you. The information will be kept confidential.

Unless required by law, only the study doctors and designee, the ethics committee and inspectors from governmental agencies will have direct access to your research records without violating confidentiality.

5) Agreements

We would like to know if you would be willing to participate in this research study. You have the right to accept or decline participation.

I agree to participate in this study and the procedures explained above.
YES ……. NO…….

Future contact:
I agree to be contacted for future studies YES…… NO……

I would like to be contacted if the genetic test results are significant
YES ……. NO…….

Using remaining blood for other future studies
We would like to keep the remaining blood samples for potential use in other future studies. There will be no extra prick. The stored blood samples will be coded (*"Coded" means identifiable, traceable. Blood and urine samples that are unidentified for research purposes but can be linked to their source through the use of codes; however, the principal investigators will be the only ones to have the list linking patients to the codes assigned.)*

I agree to permit the use of the remaining blood sample for future studies
YES ……. NO…….

If you answer YES to the above question, select one of the three options below:

I agree to permit the use of the remaining blood samples in a coded manner after contacting me for permission YES …….

I agree to permit the use of the remaining blood samples in a coded manner without contacting me for permission YES …….

I agree to permit the use of the remaining blood samples in a de-identified manner
YES …….

Your coded blood samples and data may be shared with other investigators under the supervision of the principal investigator. These investigators may be from AUBMC, from centers other than AUB, and from centers outside Lebanon.

I agree to have my coded blood samples shared with other investigators
YES ……. NO…….

I agree to have my coded data shared with other investigators
YES ……. NO…….

Investigator's Statement:

I have reviewed, in detail, the informed consent document for this research study with _____ _____ (name of patient, legal representative, or parent/guardian) the purpose of the study and its risks and benefits. I have answered to all the patient's questions clearly. I will inform the participant in case of any changes to the research study.

_____ _____
Name of Investigator or designee Signature

_____ _____
Date Time

Patient's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Nathalie Zgheib at AUB Faculty of Medicine 01-350000 ext 4846, or any of their designee involved in the study in case of any questions. If I feel that my questions have not been answered, I can contact the Institutional Review Board for human rights at 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care or benefits. I know that I will receive a copy of this signed informed consent.

_____ _____
Name of Patient or Legal Representative Signature
or Parent/Guardian

_____ _____
Date Time

_____ _____
Witness's Name Witness's Signature
(if patient, representative or parent do not read)

_____ _____
Date Time

APPENDIX B

The Role of Angiotensin Converting Enzyme Insertion/Deletion Genetic Polymorphism in the Risk, Severity and Prognosis of COVID-19 infection

Data collection sheet

Study ID:	
Date of recruitment and consent:	
Sex:	
Date of birth:	
Nationality/ethnicity:	
Date of COVID-19 testing at AUBMC:	
Reason for testing:	
History of exposure:	
Signs and symptoms upon presentation:	
Fever:	
Cough:	
Shortness of breath:	
Soar throat:	
Loss of taste/smell:	
Other:	
Smoking history:	
Comorbidities:	
List of medications:	
Lab test results:	
CBC:	
CXR:	
Other:	
Covid-19 PCR test results:	

For those who tested NEGATIVE:

Narrative of follow up history:	

For those who tested POSITIVE and NOT hospitalized:

After how many days re-tested negative:	
Detailed progression fo signs and symptoms:	
Fever:	
Cough:	
Shortness of breath:	
Soar throat:	
Loss of taste/smell:	
Other:	
Follow up lab test results:	
CBC:	
CXR:	
Other:	
Other Narrative follow up history	

For those who tested POSITIVE and HOSPITALIZED (in addition to above):

ICU admission & duration if yes	
Total length of stay until discharge	
Status at discharge	
After how many days re-tested negative?	
Total length of stay until death	Applicable for the biorepository only
Cause of death:	Applicable for the biorepository only
Heart failure	Applicable for the biorepository only
Septic shock:	Applicable for the biorepository only
ARDS:	Applicable for the biorepository only
Other:	Applicable for the biorepository only
In hospital management:	
Pneumonia &/or antimicrobials use	
Need for oxygen support & duration if yes.	
Need for mechanical ventilation & duration if	
ARDS (need for paralysis or proning)	
Cytokine release syndrome: (trt. with	
Septic shock: (trt. with pressors)	
Cardiomyopathy?	
Other:	
Narrative of follow up history:	

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