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**UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE**

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**CLINICAL FEATURES AND OUTCOMES OF SARS-COV-2
INFECTION IN RESIDENTS OF ELDERLY NURSING HOMES
WHO WERE HOSPITALIZED AT THE CLINIC OF INFECTIOUS
DISEASES, UNIVERSITY HOSPITAL OF SPLIT, IN THE PERIOD
FROM 1 MARCH TO 31 MAY, 2020**

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LIST OF ABBREVIATIONS

ACE – angiotensin-converting enzyme
ACE2– angiotensin-converting enzyme 2
ARDS – acute respiratory distress syndrome
BP – blood pressure
CDC- center for disease control
COD – cause of death
COPD – chronic obstructive pulmonary disease
COVID-19 – coronavirus disease – 2019
CoVs – coronaviruses
CRP – C-reactive protein
CT – computer tomography
CXR – chest x-ray
DNA – deoxyribonucleic acid
ER – endoplasmic reticulum
INF- interferon
IQR – interquartile range
JGA – juxtaglomerular apparatus
LDH – lactate dehydrogenase
MERS– middle-east respiratory syndrome coronavirus
MODS – multiple organ dysfunction syndrome
NT-pro BNP – N-terminal pro-B-type natriuretic peptide
ORF– open reading frames
PGE2 – prostaglandin E2
RAAS – renin aldosterone angiotensin system
RBD – receptor binding domain
RNA– ribonucleic acid
RT-PCR – real-time polymerase chain reaction
SARS-CoV – severe acute respiratory syndrome coronavirus
SARS-CoV-19 – severe acute respiratory syndrome 2019
SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2
ssRNA – single-strand RNA
TMPRSS2 – transmembrane protease serine 2
TNF alpha – tumor necrosis factor-alpha

1. INTRODUCTION

1.1 History of coronavirus

In 1962, viruses in the *coronaviridae* family were categorized as "novel respiratory tract viruses," and coronaviruses (CoVs) were not viewed as highly pathogenic in humans, causing mainly moderate respiratory and enteric infections in people with adequate immune systems (1). In 2002, in the Guangdong province of southern China, the CoVs manifested as SARS-CoV (severe acute respiratory syndrome). Its intermediary host is civet cats, and SARS-CoV spread to 29 countries, with 8096 cases and 774 deaths (2). In 2012, the Arabian Peninsula was the first outbreak of a highly pathogenic CoV with acute onset, severe respiratory symptoms named MERS-CoV (Middle-East respiratory syndrome coronavirus). MERS is zoonotic primarily through the dromedary camels endemic to the Middle East and North Africa region but can also exhibit human-to-human transmission (3). There were a total of 147 cases resulting in 63 deaths (4). We lack proper treatment and vaccination for both SARS and MERS, which has been a global concern for years.

The earliest registered case of a "*pneumonia of unknown origin*," later named SARS-CoV-19, was reported in Wuhan, China, on 12 December 2019. The virus was later named SARS-CoV-2 and the disease; coronavirus disease-2019 (COVID-19).

27 new cases were reported by 31 December. On 7 January 2020, the Chinese government isolated an unknown beta-coronavirus from bronchoalveolar lavage fluid samples. Due to the close physical location and matching medical histories to the Huanan seafood market, a zoonotic transmission is thought to have occurred. Still, some patients could not be connected to the market, indicating that human-to-human transmission was also happening (5,6).

The virus propagated quickly, and by 11 March, it had spread worldwide, at which time the WHO declared a global pandemic (5,6). Despite widespread concern, there is no proof that SARS-CoV-2 is a bio-engineered product; thus, the virus is probably novel and a result of natural mutations (7).

The first case of COVID-19 in Croatia was identified on 24 February 2020. The nationwide lockdown was implemented on 16 March. From 1 March to 31 May, the number of cases grew from 7 to 2246. The first COVID-19 fatality was on 19 March 2020, and by the end of May, the number of deaths reached 103 (8).

1.2 Virology

Origin and classification

Viruses are defined as obligate intracellular particles requiring host cellular material to replicate. We recognize two subfamilies within the *coronaviridae* family of the order *Nidovirales*: coronavirinae and torovirus. With six genera in the coronavirinae, three of them cause human illness; alphacoronavirus, betacoronavirus, and torovirus. The latter is associated with diarrheal disease. The members of alpha and betacoronavirus genera that can infect humans are listed in Table 1. In addition, several CoVs affect animals, most involving one or a few species (7,9).

Table 1. Coronavirinae subfamily overview of the alpha and betacoronaviruses.

Coronavirinae		
Genera:	Viruses affecting humans	Host receptor
Alphacoronavirus	229E	Aminopeptidase N
	NL63	Aminopeptidase N
Betacoronavirus	OC43	
	HKU1	
	ARS-CoV	ACE2
	MERS-CoV	CD26
	SARS-CoV-2	ACE2

Coronaviruses are enveloped unsegmented single-strand positive-sense RNA viruses, and they have the largest genome among the RNA viruses. Characteristic for CoVs are the distinct petal-shaped surface projections in a fringe arrangement, similar to a solar corona, hence the name coronavirus (Figure 1.) (7,9,10).

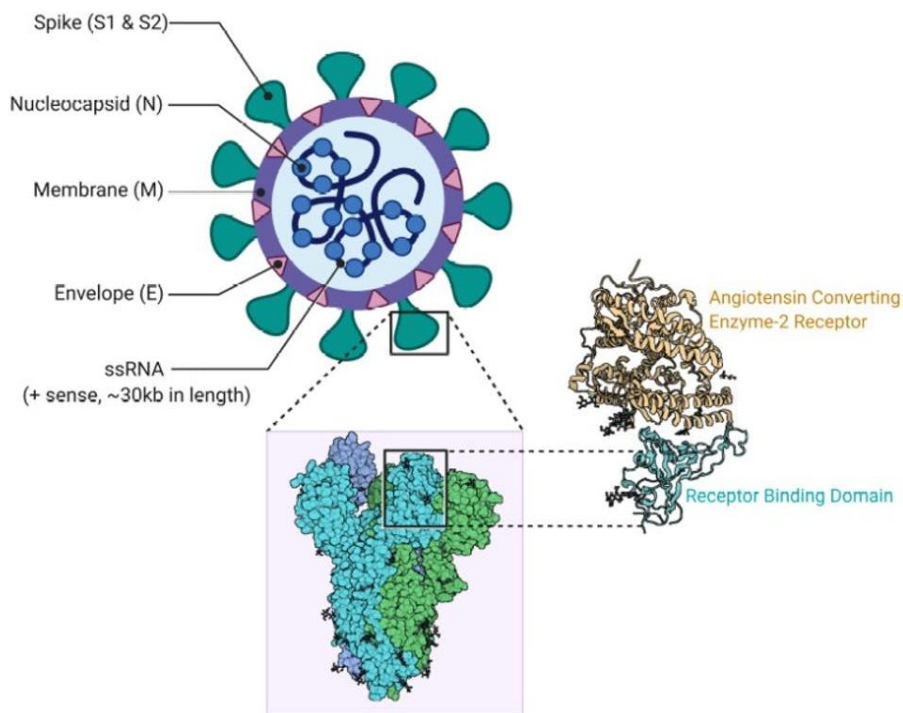


Figure 1. Structure of SARS-CoV-2. With special attention to the S protein with its receptor binding domain that attaches to ACE2.

Source from: <https://www.ncbi.nlm.nih.gov/books/NBK554776/> (10).

Genomics

SARS-CoV-2 shares 80% of its genomic sequence with SARS-CoV and 50% with MERS-CoV. It organizes its genome in the same way as other beta-coronaviruses (11,12). The genome consists of 14 open reading frames (ORF), two-thirds of which code for 16 non-structural proteins (nsp 1-16), forming a replicase complex. The remaining third codes for nine accessory proteins and four structural proteins. The structural proteins are spike (S), envelope (E), membrane (M), and nucleocapsid (N). Proteins encoded by SARS-CoV and SARS-CoV-2 have mostly the same length, and the structural genes share 90% similar amino acid identity. The divide is within the S-gene coding for 16 structural proteins involved with transcription and replication, resulting in 85% similarity between the two viruses (11,13).

Phylogenetic analysis clusters SARS-CoV-2 with SARS-CoV and SARS-related viruses. It is placed in subgenus Sarbecovirus in the betacoronavirus genus among four horseshoe bat coronavirus isolates and a novel coronavirus newly discovered in pangolins. Even though it is phylogenetically related, SARS-CoV-2 is distinct from all other bat and pangolin coronaviruses (11).

The S-protein is longer than that of SARS-CoV and bat coronaviruses. A distinct genetic feature of SARS-CoV-2 is the four amino acid residue insertion (PRRA) at the subunit S1 and S2 junction of the S-protein. The insertion generates a polybasic cleavage site (RRAR), enabling effective cleavage of proteases, especially furin. This cleavage site is not

present in other viruses, but a similar three amino acid insertion is found in a specific bat-derived coronavirus, substantiating the belief that this can occur naturally. Structural studies suggest that the "furin-cleavage site" can cause the S-protein instability, thus facilitating the conformational adaptation necessary for a receptor-binding domain (RBD)-receptor binding. The RBD of SARS-CoV-2 shares only 73% similarity with SARS-CoV (11,13).

The accessory gene *orf8* of SARS-CoV-2 is another difference. This gene encodes a novel protein with only 40% amino acid similarity with that of SARS-CoV. This changed gene lacks the motif that triggers intracellular stress pathways and may indicate human adaptation after animal host transmission. This variant was widespread in Singapore in the early pandemic and resembles late phase nucleotide deletions in the ORF8-region observed in the 2002-2003 SARS-CoV outbreak (11).

1.3 Pathogenesis

Cellular entry and replication

The S-protein on the viral envelope can bind to specific receptors on the host cell membrane. The SARS-CoV-2 uses ACE2 as its receptor, and its binding affinity to ACE2 is 10-20-fold higher than SARS-CoV. The S1 subunit containing the RBD binds to ACE2, and structural rearrangement exposes the "furin cleavage site" on the S2 domain, enabling viral entry by fusion after shedding of S1. The cellular entry and replication are visualized in Figure 2. The transmembrane protease serine 2 (TMPRSS2) cleaves the S-protein and is responsible for its activation before the cell fusion. Endolysosomal cathepsin-L mediates virus-cell membrane fusion at the cell surface and endosomal compartments and can compensate for entry in cells lacking TMPRSS2 (13).

To have a viable infection, intact ACE2 or its transmembrane domain is internalized along with the virus. The S-protein binding process does not hamper the catalytically active site on ACE2; therefore, this activity is independent of its peptidase activity in the renin-angiotensin-aldosterone system (RAAS). ACE2 is expressed on alveolar epithelial cells and weakly on the epithelial cell surface of nasal, oral, and nasopharyngeal mucosa; this suggests that the lungs are the primary target. However, ACE2 is also prominent throughout the rest of the body, such as myocardial cells, proximal tubule, bladder urothelial cells, enterocytes, and blood circulation (14–18).

In the cytoplasm, the positive sense ssRNA utilizes the host ribosome to produce polyproteins. It also uses RNA-dependent RNA polymerases in order to duplicate its RNA. ORF1a and ORF1b are translated by viral replicase proteins and then cleaved into individual

nsps via both host and viral proteases. The result is RNA-dependent RNA polymerase. Then replicase components arrange the endoplasmic reticulum (ER) into double-membrane vesicles, facilitating viral replication of genomic and subgenomic RNAs. The latter is then translated into accessory viral structural proteins, ultimately forming the viral particle (13).

Progression of viral infection

When SARS-CoV-2 has entered the host via the respiratory tract, airway, and alveolar epithelial cells, vascular endothelial cells and alveolar macrophages are among the first cellular targets. Paradoxically, the expression of ACE2 in the lung is relatively low compared to the extrapulmonary tissues leading to a hypothesis that viral entry may heavily depend on the expression of TMPRSS2 (13,18).

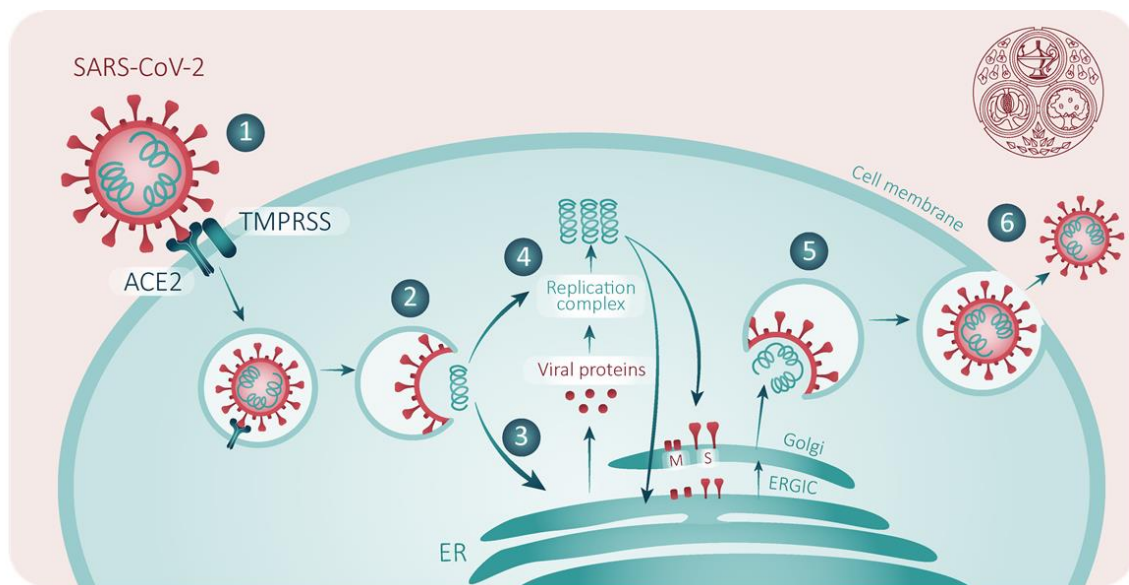


Figure 2: 1. Spike protein on the virion binds to ACE2. TMPRSS2 enzyme helps virion enter. 2. The virion releases its RNA 3. Some RNA is translated into proteins by the cell. 4. Some of the resulting proteins form a replication complex to make more RNA. 5. Proteins and RNA are assembled into a new virion in the Golgi apparatus. 6. Released. *Source from: <https://www.fpm.org.uk/blog/covid-19-sars-cov-2-pandemic/> (18)*

Role of ACE2

The RAAS is essential in maintaining blood pressure and fluid and electrolyte homeostasis. A schematic overview is provided in Figure 3. The kidney release renin from its juxtaglomerular apparatus (JGA) in response to perceived low blood pressure (BP). Low body fluid volume can be indicated through arterial baroreceptors, decreased amounts of sodium chloride detected by the macula densa, or sympathetic activity detected by betaadrenergic receptors. Renin cleaves angiotensinogen from the liver to produce the decapeptide angiotensin I (Ang-I). Angiotensin-converting enzyme (ACE) removes two amino acids, resulting in the octapeptide angiotensin II (Ang-II).

The ACE enzyme is present primarily on endothelial cells of the lung vessels. Ang-II is active and works to rectify this perceived volume/pressure loss. It has two primary receptors: AT1R and AT2R, the latter receptor antagonizing the first's actions. Through the AT1R, the Ang-II causes vasoconstriction, extracellular matrix remodeling, coagulation, pro-inflammatory actions, and oxidative stress. Thus, Ang-II widely contributes to the progression of cardiovascular diseases, hypertrophy, fibrosis, atherosclerosis, cell death, and arrhythmia.

Angiotensin-converting enzyme 2 (ACE2), a homolog of ACE, can cleave Ang-II yielding the heptapeptide angiotensin-(1-7). ACE2 or other endopeptidases and oligopeptides can also cleave Ang-I converting it to angiotensin-(1-9), although this is an accessory pathway. Angiotensin-(1-7) exerts its functions through the G-protein-coupled receptor MAS and works mainly to oppose Ang-II's main actions. ACE2 is also involved in amino acid uptake in the intestinal epithelial cells. SARS-CoV-2 infection can downregulate ACE2

expression on cells, thereby disrupting the ACE/ACE2 ratio and Ang-II/angiotensin (1-7) relationship, leading to organ injury through the AT1R (17,19).

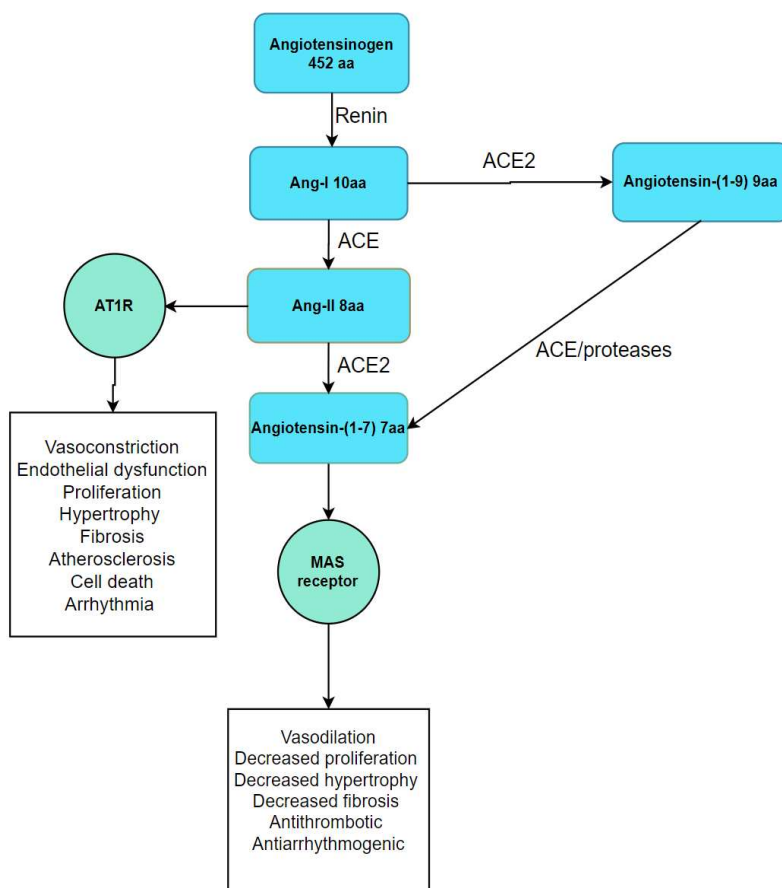


Figure 3: A schematic overview of the RAAS and its effects on tissues.

Inflammation

The ACE-2 dependent cellular entry of the SARS-CoV-2, as described above, triggers T-helper cells and synthesis of interferon-gamma (IFN-gamma), further resulting in inflammatory cell mobilization and cytokine storm. The cytokine storm can then cause the organ failure and multiple organ dysfunction (MODS) seen in severe cases (20).

The virus also releases specific inflammatory mediators that stimulate macrophages to release cytokines (IL-1, IL-6, and TNFalpha) and chemokines (CXCL10 and CCL2) into the circulation. These molecules cause vasodilation and increased capillary permeability. In the lungs, this leads to plasma leakage into the interstitial space causing accumulation of fluid around the alveoli and compression, ultimately causing atelectasis and impaired gas exchange. The hypoxic state can stimulate the sympathetic system resulting in tachycardia. At the same time, there is a cytokine storm due to the increased release of inflammatory cytokines. CD4+ helper cells increase the production and recruitment of neutrophils and macrophages through IL-17, IL-21, and IL-22. In later stages, these steps contribute to dyspnea, hypoxemia, and cough (21).

Fever is due to prostaglandin release (PGE2) by IL-1, IL-6, and TNF alpha effect on the hypothalamus. These mechanisms causing hypotension and decreased vascular resistance can also ultimately cause septic shock and multiple organ failure (21).

1.4 Epidemiology

Transmission

Disregarding the initial poorly understood mechanism of interspecies transmission, the essential mechanism propelling the pandemic is the human-to-human aerosol transmission with infective droplets. Bats are postulated to be natural hosts. The virus is proven to persist on metal, plastic, and glass surfaces for several days, although it can be inactivated with proper disinfection practices. Most evidence indicates that maintaining social distancing of 1.5m is enough to prevent airborne transmission (22). Leung *et al.* show that surgical masks can effectively reduce the emission of viral particles into the environment through respiratory droplets ($>5\mu\text{m}$). Therefore, mask use by potentially sick people is most helpful to prevent outward spread (23). Transmission is possible approximately eight days post symptom appearance but is also proven in the absence of symptoms. The contagiousness of infected individuals is highly variable. Real-time polymerase chain reaction (RT-PCR) tests may remain positive for several weeks, even after symptom remission and non-viable virus. Thus, prolonged RT-PCR positivity is not a good indicator of clinical transmission.

Therefore, it is logical to suggest that isolation is unnecessary after two consecutive negative RT-PCR at an interval of at least 24h while in the absence of clinical or epidemiological criteria (22).

The R_0 for SARS-CoV-2 is approximated to 2-3 although, various estimates on the asymptomatic proportion hamper this calculation (21).

Incubation period

Incubation time is defined as the period of time from infection to illness onset. It is vital to know the incubation period of infectious diseases because, during this time, people might unknowingly spread the infection. The quarantine period should cover this incubation period to ensure that a potential illness emerges during the time of seclusion (24). In a study of 1099 Chinese patients with laboratory-confirmed symptomatic COVID19, the incubation period was shown to be four days with an interquartile range of 2-7 days (25). Another study with 181 participants looked at the median incubation period and within which time duration the majority will develop symptoms. They established that the median incubation period is about 5 days and that 97,5 % of infected will develop symptoms within 2-14 days (26). Most countries initially operated with quarantine periods of 10-14 days with varying regulations of shorter duration if there is evidence of one or multiple negative RT-PCR tests.

1.5 Clinical manifestations

The clinical features of COVID-19 range from an asymptomatic condition to an acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), leading to death. Some studies have organized the disease progression into three stages, shown in Figure 4.

Stage one, early infection, is asymptomatic or mildly symptomatic during the initial 1-2 days. The inhaled virus binds to epithelial cells of the nasal cavity and starts replicating. There is a local spread of the virus but a limited innate immune response. The virus is detectable in the nasal swab at this stage, and the subjects are infectious despite low viral loads. Mild constitutional signs and symptoms might arise, such as fever, dry cough, and lymphopenia. Patients limited to the first stage of the disease has no need for hospital care and excellent prognosis and recovery (15,27)

Stage two, the pulmonary phase, is the duration of the next few days when the upper and conducting airway respond. In this period, the virus replicates and spreads down the respiratory tract and triggers a more robust immune response. The disease will manifest clinically. Viral infected epithelial cells constitute a significant source of beta and lambda

INFs. Determining the host's innate immune response might allow for predictions about progression and monitoring needs. In the majority (80%), the disease will be mild and mostly restricted to the upper and conducting airways, and these can have home-monitoring with symptomatic treatment (15,27).

Stage three is the hyper-inflammation phase characterized by hypoxia, ground-glass infiltrates, and progression to ARDS. These constitute the remaining 20% that will develop severe disease. The virus has reached the lung periphery and interferes with the gas exchange, preferentially infecting the type two pneumocytes. Replication within the type 2 pneumocytes causes a large number of particles to be released, and the cells undergo apoptosis. In this way, whole lung areas will lose most of their type 2 cells, and as a precursor to type 1 cell, these numbers will also diminish. This will trigger a secondary pathway for epithelial regeneration. The result is diffuse alveolar damage with fibrin-rich hyaline membranes and some multinucleated giant cells. The aberrant wound healing may result in more severe scarring and fibrosis than other types of ARDS. Recovery demands vigorous innate and acquired immune response and epithelial regeneration (15,27).

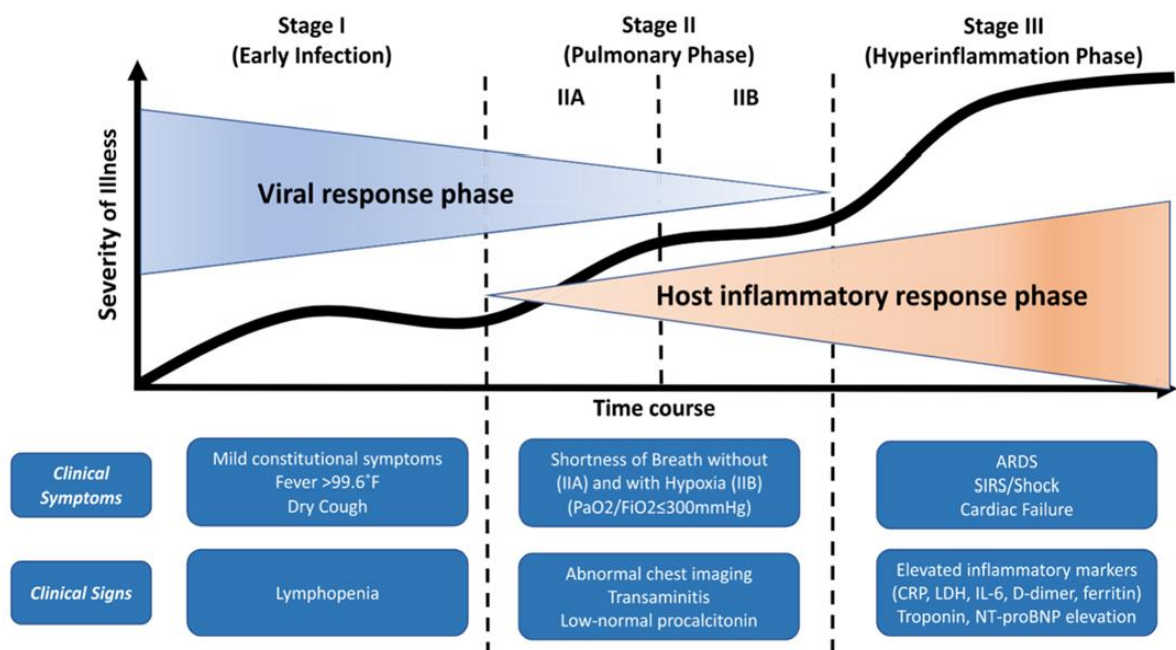


Figure 4. Classification of COVID-19 disease states. The figure illustrates three escalating phases of COVID-19 disease progression, with associated signs, symptoms.

Source from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7118652/> (27)

1.6 Diagnostic tests and imaging characteristics

Diagnostic tests

In order to properly diagnose infectious diseases, the gold standard remains to establish the presence of the infectious agent. To demonstrate an etiological diagnosis, we can perform direct or indirect testing. A direct test targets the infectious agent's genetic material, while an indirect test targets the body's response (28).

The gold standard for diagnosing COVID-19 is direct with RT-PCR. Early genomic sequencing of the virus and identifying specific regions in the genome, and the subsequent manufacturing of matching probes ensured this method's quick initiation. Reverse transcriptase is used to transform viral RNA into complementary DNA. Then the specific regions are amplified, and probes and primers are added so replication can happen. Multiple serial amplification cycles are performed until the targets are identified. The more cycles needed, the lower the viral load. Several different kits with varying sensitivity and specificity are available. Despite being the gold standard for diagnosing, RT-PCR has a sensitivity of about 70% and specificity of 95%. This causes concern about false-negative results, and a negative RT-PCR test does not preclude infection if there is clinical suspicion (28).

It is recommended to collect an upper respiratory specimen, a nasopharyngeal being preferred. Other alternatives like oropharyngeal, nasal-mid turbinate specimen, nasal swab, or a nasopharyngeal wash/aspirate specimen can be used (29).

This diagnostic method is vulnerable to mistakes and variability at every testing level, from collecting to transportation and in the lab. The virus can also mutate so that the target sequences are different, and the test will be unable to detect the new variant, leading to false-negative results. In addition, a general mismatch between the primers and probes may result in false-negative results. This is mitigated by amplifying more than one viral genome region per test (often about four). A low viral load, such as in very early disease or sometimes asymptomatic individuals, can also elicit a false-negative result due to the low amounts of RNA not being amplified enough to get a signal in the standard number of amplification cycles. A false-positive result is most commonly due to improper handling leading to contamination at the collection stage.

In the early period of the pandemic, quick tests and serological tests were not commercially available.

Imaging characteristics

Chest imaging may be normal in early or mild disease. When abnormalities are present, they often manifest as consolidation and ground-glass opacities distributed bilaterally in the lower lung fields, and lung involvement characteristically increase throughout the disease. The peak of radiological severity manifests at 10-12 days after symptom onset (30). Although chest tomography may be more sensitive than chest x-ray (CXR), the findings are not specific enough to confidently include or exclude SARS-CoV2. The findings are primarily characteristic of viral pneumonia. Therefore, the American College of Radiology advises using CT and CXR for hospitalized patients only when necessary for the management and excluding screening and diagnosis (31). Pulmonary ultrasonography typically shows B-lines, consolidations, and pleural thickening. Its advantages are good sensitivity, lack of radiation, and easy to perform without transportation or sedation (28).

1.7 Demographics

Age

Studies show that older age, even in the absence of other health difficulties, is associated with increased mortality in SARS-CoV-2 infection. In a Glasgow cohort study by Ho *et al.*, participants aged over 75 without additional risk factors had a 4-fold increase in mortality risk than similar patients under 65 years. Patients over 75 with additional risk factors had 13 times increased mortality risk to those under 65 years of age (95%CI 9.13-17.85). 39.3% of their excess risk could be explained by: low FEV1, high systolic BP, low handgrip strength and, multiple long-term conditions. Healthy older adults were at substantially lower risk; nevertheless, older age was an independent risk factor for mortality (32). A meta-analysis of 33 studies with 3027 participants showed that adults over 65 years were at five times increased risk of becoming critical or die (1 in 23) (33).

Regular age-related decline in respiratory function and diminished clearance of inhaled particles in the small airways are possible factors responsible for the high prevalence of respiratory symptoms among the elderly. Both cilia and upper airway sizes decreased with increasing age in both genders, although men showed greater upper airway collapsibility. Other factors are weaker immune response, obesity, and comorbidities (34).

Younger people may be more prone to get infected, but studies suggest that the lower levels of ACE2 in the elderly may contribute to a more severe clinical course. It has been observed that patients with more aggressive courses are often in the older age groups and may progress towards ARDS (32).

Nursing homes

COVID-19 infection does not infect all societal groups equally. People in nursing homes are particularly exposed, reflecting the number, mortality, and severity of the infections. Staff may bring the infection with them from home or their commute and from room to room. Often these facilities can be crowded with several residents per room, and it is challenging to implement social distancing correctly. Added factors are the abundance of patients with cognitive decline that may not understand the situation and are prone to wandering. It is also a group of people with advanced age, multiple comorbidities, and immunological dysfunction, which by itself can increase susceptibility to infection.

A Canadian study looked at risk factors associated with mortality in COVID-19 positive residents at long-term care facilities. They found a 13-fold increase in incidence rate ratio for COVID-19 related deaths in the residents compared to the general population over 69 years, further substantiating that residents of long-term care facilities are particularly vulnerable even discounting their age (35).

1.8 Comorbidities

Diabetes mellitus type 2

The high prevalence of diabetes mellitus among the world's population, especially among the elderly and people with other health issues, lead to an early interest in this subgroup. Diabetic patients of all types are generally at increased risk of infection due to dysregulation of the innate and adaptive immunity responses. The vascular and metabolic changes observed in the diabetic syndrome can also affect the body's response to pathogens (10). Insulin resistance and hyperglycemia are known to increase the production of pro-inflammatory cytokines, glycosylation-end-products and stimulate the synthesis of adhesion molecules that mediate tissue inflammation and contribute to oxidative stress. Still, these mechanisms are not fully understood, and there is a possibility that the susceptibility may be due to the associated cardiovascular, renal comorbidities, and old age (24).

ACE-2 receptors are present on pancreatic islet cells, and SARS-CoV-2 infection is proven to cause hyperglycemia in patients with no pre-existing diabetes condition (20). Furthermore, Guo *et al.* linked dysregulation of glucose metabolism with uncontrolled inflammatory responses and a hypercoagulable state as well as significantly higher serum levels of inflammatory markers and coagulation markers. This substantiates the belief that people with diabetes are more susceptible to an uncontrolled cytokine storm resulting in rapid deterioration (36).

Studies suggest that diabetes is often associated with severe disease, ranging from 14-32%. A review of available data experienced increased incidence and severity in diabetics (20). Wu *et al.* found a significant difference between non-severe and severe COVID-19 cases (37). Bode *et al.* observed that patients with diabetes and uncontrolled hyperglycemia had an extended stay in hospital and markedly higher mortality, 41.7% compared to 14.18% for other patients (38). The Chinese Center for Disease Control and Prevention made a summary report of 44 672 infected patients, showing a case fatality rate of 2.3% in the general population, compared to 7.3% in diabetics (6).

Cardiovascular disease

There is a bidirectional relationship between cardiovascular diseases (CVD) and COVID-19, where patients with prior CVD are more prone to severe clinical manifestations, which can exacerbate their underlying cardiovascular issues, resulting in a vicious cycle. However, it is still unknown whether prior CVD impacts susceptibility to SARS-CoV-2 infection (39).

The center for disease control (CDC) reported a 9% CVD prevalence among the patients. Different CVD subtypes among the patients ranged from 2.5-27.7 %, with heart disease 0.8-21% for heart failure and 1-19% for atrial fibrillation. A systematic review and meta-analysis pooled data from 300 000 patients with prior CVD ranged over ten studies. They showed a five-fold increased risk of severe COVID-19. In addition, a few studies reported an independent association of CVD with severe COVID-19 after correcting for confounding factors (39).

The impact on the cardiovascular system varies from direct cardiac damage to thrombotic events. 60-70% of hospitalized patients have abnormal ECG findings, and elevated cardiac markers are found in 7-36%. It is well known that COVID-19 may interfere with the coagulation system. The incidence of venous thrombosis and pulmonary embolism has been reported to be increased. In a systematic review of 20 studies with 1988 participants, venous thromboembolism was recognized in 31.3%. There are also reported arterial thromboses such as stroke and myocardial infection (39).

Hypertension

Hypertension is a widespread disease estimated to affect about 1.39 billion people worldwide, and it is known to be the most prevalent comorbidity among COVID-19 patients. Previous studies concerning SARS-CoV and MERS identified hypertension as a risk factor for increased mortality (40), though there is controversy regarding its classification as a risk

factor for susceptibility and severity in COVID-19 infection. If we compare Zhou *et al.* study with a Chinese nationwide survey with similar age distribution, the prevalence of hypertension is 30.4% and 44.6%, respectively. Meaning higher among the general population (41,42). The same tendency was observed in Italy and the US. There no substantial evidence suggesting that those with hypertension have increased susceptibility for SARS-CoV-2 infection (40).

At first glance, several studies report an association between hypertension and disease progression and severity, though this did not persist after adjusting for age and sex (43). However, the most extensive epidemiological study to date, examining over 17 million health records in England, after adjusting for confounding factors, suggests that hypertension (or recorded BP \geq 140/90 mmHg) is not associated with COVID-19 in-hospital severity (hazard ratio (HR) 0.95, (95% confidence interval (CI) 0.89–1.01) (44). In addition, the American CDC does not include hypertension in its list of risk factors for severity (45).

Neurological disease

Both viral and autoimmune meningoencephalitis have been reported in patients with COVID-19, although these complications are quite rare (46).

Aside from the risk due to living environments (nursing homes or generational homes), the effects of dementia, especially memory loss, makes living in a pandemic situation particularly challenging. An estimated 50 million people live with dementia, which is expected to double every 20 years. Due to cognitive decline, they may struggle with understanding and complying with disease precautions (21).

Patients with debilitating neurological diseases like amyotrophic lateral sclerosis and multiple sclerosis are likely to develop a more severe course and may not return to baseline.

At this time, there is no conclusive evidence to suggest that patients with a neurologic disease who are treated with immunosuppressants are at increased risk of COVID-19, nor that their prognosis is worse if infected. Therefore, although there should be more research on the topic, it is not recommended to change their treatment regime, discontinuing on an individual basis only if the infection develops (47).

Chronic lung disease

The most important cause of death in patients with COVID-19 is respiratory failure due to pneumonia, which means COVID-19 can be expected to be more frequent and severe in people with chronic lung disease such as moderate to severe asthma, COPD, cystic fibrosis, and pulmonary hypertension. Zheng *et al.* studied 3027 patients in their meta-

analysis, concluding that previous respiratory disease was significantly higher in critical and mortal patients compared to the non-critical patients (33). In addition, the most critical risk factor for COPD is smoking, which is also an independent risk factor for COVID-19 infection (48).

Asthma does not seem to increase the susceptibility of COVID-19 infection, and studies could not identify that people with asthma had an increased risk of becoming seriously ill. Nevertheless, it is still vital to maintain good asthma control, as poorly controlled asthma may lead to a more challenging course, and some studies have found a higher rate of intubation and prolonged mechanical ventilation in adults with asthma (49,50).

Malignancy

Increasing evidence suggests that cancer patients are at higher susceptibility, morbidity, and mortality than the general population. Thus far, data from China have shown that cancer patients infected with COVID-19 are at 3.5 times increased risk of requiring mechanical ventilation or ICU admission compared to the general population. Other factors associated with malignancy, such as diminished immune system, age, and other comorbidities, will also affect the susceptibility and severity of SARS-CoV-2 (51). Blood cancers may increase the risk of prolonged infection and severity compared to people with solid tumors. Patients with leukemia and lymphoma often have abnormal or diminished numbers of antibody-producing cells, which is believed to be the reason for the increased risk. Meng *et al.* report a history of cancer as an independent risk factor for mortality in COVID-19 infection (52).

3. OBJECTIVES

The aims of the study were:

1. To determine the proportion of asymptomatic and symptomatic forms of infection;
2. To determine mortality according to age;
3. To determine mortality according to comorbidities;
4. To establish the proportion of patients who died from respiratory complications (including pulmonary thromboembolism) and patients who died from other complications;
5. To determine time of PCR negativization.

We developed five hypotheses:

1. The proportion of symptomatic infections among nursing home residents was higher compared to data from the general population;
2. Among elderly patients, COVID-19 mortality also increases with older age;
3. The presence of two or more comorbidities was associated with a more severe clinical feature of the disease and higher mortality;
4. The leading cause of death was respiratory complications COVID-19;
5. The time of PCR negativization is longer than in the general population.

4. SUBJECTS AND METHODS

Ethical approval

The research protocol was reviewed and approved by the Split Ethical Committee.

Study population

This study included 94 SARS-CoV2 positive nursing home residents treated at the Clinic of Infectiology at University Hospital of Split from 1 March to 31 May 2020. The patients were either admitted with a positive RT-PCR test or tested positive during their stay. Exclusion criteria were if the patient records were unavailable or incomplete. In the observed period, all residents of nursing homes with a proven SARS-CoV2 infection were hospitalized, regardless of whether the infection was asymptomatic or symptomatic.

Study design

This research was conducted as an observational retrospective study.

Method for collecting and analyzing data

The medical data from the eligible patients were retrieved by collecting patient information from records stored in the archive at the Split Clinic of Infectiology.

The collected medical data includes the following: 1) demographic information such as age, gender, and living situation, 2) duration of hospital stay, 3) severity, 4) if they died in hospital and proposed mechanism or cause of death, 5) duration from the first positive PCR test to the first of two consecutive negative tests, 6) the number of comorbidities.

Severity was established by separating the participants into five groups according to the clinical features; 1) asymptomatic infection, 2) acute respiratory infection without pneumonia, 3) pneumonia without the need for oxygen treatment, 4) pneumonia requiring oxygen treatment, 5) pneumonia that requires mechanical ventilation.

We determined the number of comorbidities by categorizing the comorbidities into main groups such as cardiovascular disease (hypertension, chronic heart disease), malignant diseases, chronic kidney disease, chronic liver disease, immunosuppressed patients, chronic neurological disease, diabetes mellitus, and chronic lung disease. Then we classified the patients into two sections according to the number of groups: 1) patients with one comorbidity, 2) patients with two or more comorbidities.

The duration of illness was defined by counting the days between the first positive RT-PCR test to the first of two consecutive negative tests. If the patient tested positive at an undetermined time before admission, the date was set as the day of admission. For whatever reason, the participants that had no evidence of a negative test during their stay were not counted in the duration table.

Categorical variables are reported as percentages and proportions. Continuous variables are reported as mean, median, range, and interquartile range values. Using chi-square tests, we could compare the categorical variables age and mortality, comorbidities and mortality, and comorbidities and severity. A *P*-value less than 0.05 was considered statistically significant.

We used the *Chi-Square Calculator for 5x5 (or less) Contingency Table* from *SocialScienceStatistics* (53).

5. RESULTS

The ages of the 94 participants range from 47 to 96 years. There were 15 men and 79 women, constituting 16 and 84%, respectively. Table 2 divides the patients who died into six age groups, ranging from 45-54 to 95-104. The highest number of patients were found in the 75-84 age range (36), while the 45-54 and 55-64 age range only had 1 and 4, respectively. The age-specific mortality rate per 100 was calculated, and Figure 5 graphically represents Table 2, with the age-specific mortality rate plotted against the age groups. There is an upward trend after the 65-74 group. Before using a Chi-square test to determine the association between age and mortality, we excluded the category 45-54 because it only contained one patient with resulting mortality of 100%. The test result showed $\chi^2(3, N=93) = 10.21, P=0.037. P<0.05$ indicating that there is a statistical significance in the relationship between age and mortality.

Table 2. Mortality according to age groups.

Age group (years)	Deaths N	Total per group N	Age-specific mortality rate per 100
45-54	1	1	100
55-64	1	4	25
65-74	1	13	7.69
75-84	6	36	16.67
85-94	14	35	40
95-104	3	5	60
Total	26	94	27.66

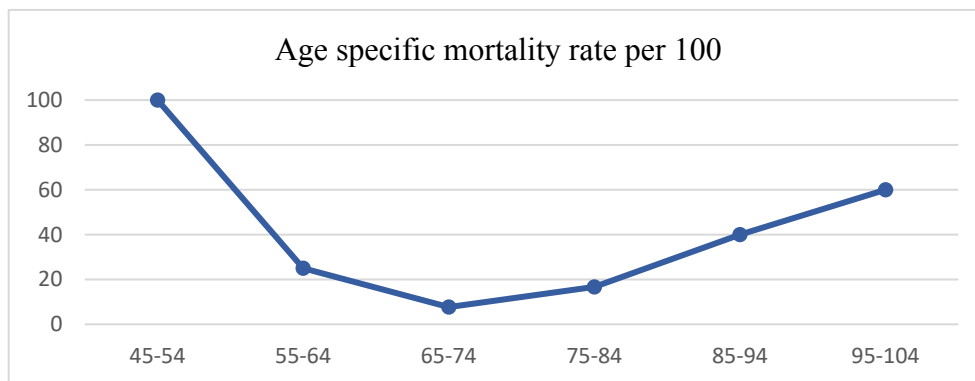


Figure 5. Age specific mortality rate per 100 is plotted against the age groups showing upward trend after the 65-74 group.

Table 3 stratifies the non-survivors into two groups according to their number of comorbidities, "one" and "two or more". Then we calculated the specific death rate per 100 for each group, 24.14 (one comorbidity) and 29.13 (two or more comorbidities). The Chi-square test was used to determine a significant relationship between the number of comorbidities and mortality. $\chi^2(3, N=94) = 0.30, P=0.610$. The result is not significant at $P<0.05$.

Table 3. Mortality according to the number of comorbidities.

	Dead N	Alive N	Specific death rate per 100
Comorbidity 1	7	22	24.14
Comorbidity 2+	19	46	29.13
Total	26	68	

To explore the relationship between severity of infection and number of comorbidities, we divided the patients among the two groups of comorbidities into one of five severity classifications (Table 4). The percentages are shown in parenthesis after the number of patients in each group. Figure 6 visually represents the proportion of patients from each comorbidity group in their severity classification. Clinical 4 (pneumonia requiring oxygen) had the highest percentages in both comorbidity groups at 34% and 32%. The standard error is in intervals at the ends of the columns. All the standard error intervals overlap except in the clinical 2 group (acute respiratory tract infection without pneumonia). The Chi-square test resulted $\chi^2(3, N=94) = 2.06, P=0.724$. The P -value is over 0.05, meaning that we could not prove a statistically significant relationship between severity and number of comorbidities.

Table 4. Severity according to number of comorbidities

	clinical 1	clinical2	clinical 3	clinical 4	clinical 5	Total
	N, (%)	N, (%)	N, (%)	N, (%)	N, (%)	N
Comorbidity 1	4 (14)	7 (24)	5 (17)	10 (34)	3 (10)	29
Comorbidity 2+	11(17)	9 (14)	13 (20)	21 (32)	11 (17)	65

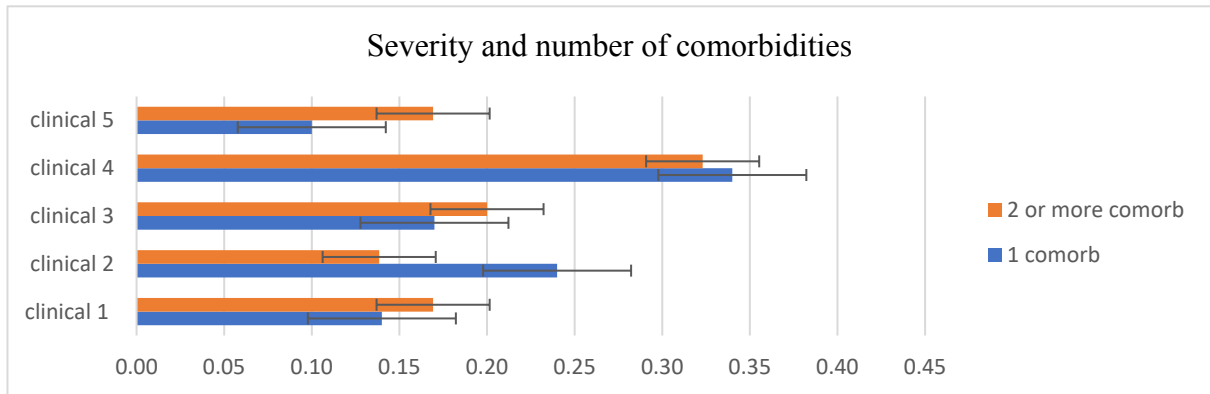


Figure 6. The proportions of patients stratified into the number of comorbidities are arranged according to severity. The standard error is the intervals at the end of the columns.

Figure 7 shows the proportion of symptomatic to asymptomatic patients, 0.84 and 0.16, respectively. The symptomatic group was over four times larger than the asymptomatic group. The cause of death is similarly expressed in Figure 8 as the proportion of cause of death (COD) attributed to respiratory complications, and COD credited to other than respiratory complications. Respiratory COD was in the majority with 81%.

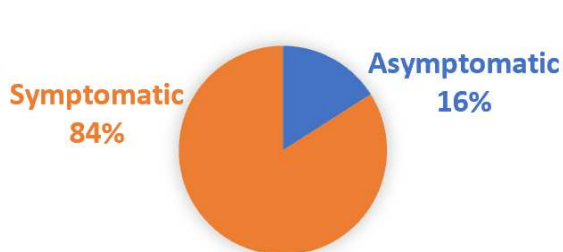


Figure 7. The data from Table 5 are presented as percentages showing the asymptomatic and symptomatic number of participants.

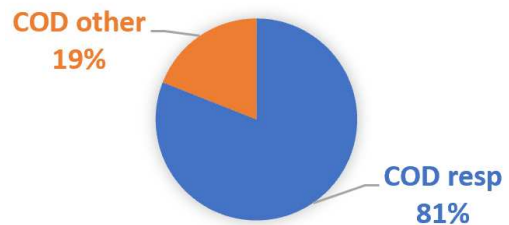


Figure 8. The data from Table 6 are presented as percentages showing the respiratory and other causes of deaths among the participants.

Of 94 participants, 72 had relevant information about testing. Table 5 is an overview of the time of PCR negativization expressed in days. The range was 3-56 days with a mean of 21.67, median of 19.5, and interquartile range of 15-27 days.

Table 5. Time of PCR negativization

	Days between positive and negative tests
Mean	21.67
Range	3-56
Median	19.5
IQR	15-27

6. DISCUSSION

Hypothesis 1: the proportion of symptomatic infections among nursing home residents was higher than that of the general population

Regarding the first hypothesis, our results showed that the study population involved 84% symptomatic with resulting 16% asymptomatic.

In the general population, a person is naturally more likely to be tested if they produce symptoms, which leads researchers to be cautious in referencing the percentage of asymptomatic infections without a substantial margin of error. These patients are also more likely to be entered into research. This aspect is slightly mitigated in the nursing care homes in our study, where we can assume everyone in the facility was tested if anyone contracted the disease. All residents who tested positive from nursing homes in Split and the vicinity were admitted regardless of symptoms. The majority were not discharged until after the two consecutive negative tests, meaning that as long as there are no symptoms added in their files, we can assume they remained asymptomatic. Therefore, our results should be similar to the actual proportion unless their previous health issues masked mild symptoms.

Early studies aiming to determine the proportion of asymptomatic infections in the general population had huge heterogeneity with percentages ranging from 3% to 83% (54). Recent studies have discussed the terminology; asymptomatic and significantly mild symptomatic versus presymptomatic infections. They argue that a considerable proportion of those we earlier thought of as asymptomatic are experiencing very mild symptoms or are presymptomatic. A significant flaw of the early studies is that they had very different methodologies and follow-up before labeling someone as asymptomatic. One week after the first positive test is not sufficient to determine that they never developed symptoms.

Concerning people with very mild disease, one could argue that as long as they are not identified as having COVID-19 symptoms or realize this for themselves, the effect is the same as for the asymptomatic patients. They will not be identified for testing unless they are in close contact with someone who developed symptoms or performed an activity requiring testing and can transmit the virus without knowing. Moreover, if the duration of presymptomatic infection is incredibly long, the outcome would also model the asymptomatic.

Upon reviewing the issue on asymptomatic transmission, Pollock *et al.* stated that the initial belief that the asymptomatic percentage was 80% is too high and has since been revised down to about 17-20%. They also remarked on the heterogeneity and incomplete symptom assessment of the earlier studies. 49% of the people initially defined as

asymptomatic went on to develop symptoms, which may be one of the causes of the high initial numbers reported (55). A metaanalysis and systematic review, merging data from 41 studies with a total of 50 155 patients, determined that the pooled percentage of asymptomatic SARS-CoV-2 infected patients was 15.6% (95%CI 10.1%-23.0%) (56). Accounting for bias in their 79 included studies, a systemic review, and meta-analysis by Buitrago-Garcia *et al.* estimated that the proportion of patients not developing symptoms during their infection was 20% (95%CI 17-25) (57).

Thus, taking the current research into account, we cannot establish any meaningful difference between our results. However, further studies are needed in order to provide credible evidence.

Hypothesis 2: among elderly patients, COVID-19 mortality also increases with older age

Concerning our second hypothesis, among elderly patients, COVID-19 mortality also increases with older age. Our results showed an upward trend after the age group 65-74 years, and the relationship between age and mortality was statistically significant with a *P*-value of 0.037. The two youngest age groups, 45-54 and 55-64, contained the least participants, 1 and 4, respectively. One participant from each of these groups died, resulting in age-specific mortality rates of 1 and 0.25, which is not representative for this age population, and was considered outliers. The residents at nursing homes in Croatia are generally older than 64 years, and these participants likely had other health issues that prevented them from managing on their own, which may have contributed to the increased mortality observed among this small subgroup.

Several studies support our findings with increasing age leading to higher mortality. In their cohort study of 470 000 participants, Ho *et al.* showed that mortality increases with age and that the association is exponential. For example, patients aged 75 or above were at a 13-fold increased mortality risk than those aged less than 65 (32). Furthermore, multivariate regression performed by Zhou *et al.* showed that the increased odds of in-hospital death are associated with older age and increases with each year (OR: 1.10, 95% CI 1.03–1.17, per year increase *P*=0.0043) (42).

As of 18 June 2021, the county of Split-Dalmatia has had 45 315 cases with 623 deaths, resulting in a mortality rate per 100 of 1.37 (58). This mortality rate is about 20 times lower than the general result in our study of 27.66. This indicates a tremendous difference in mortality between the local general population and those living in elderly nursing home facilities.

In conclusion, the result that mortality increases with age has been observed throughout the pandemic by multiple sources, and we can confidently conclude that our results fall in this line.

Hypothesis 3: the presence of two or more comorbidities was associated with a more severe clinical feature of the disease and higher mortality

On the third hypothesis, exploring the relationship between the number of comorbidities and severity and mortality, our study had likely too few participants stratified into several groups. Consequently, we could not prove a statistically significant correlation between the number of comorbidities and severity ($P = 0.724$) and mortality ($P = 0.610$). Perhaps a larger sample size could have led to significant results. Furthermore, the lack of participants without comorbidities and our inclusion of several asymptomatic patients made it challenging to elucidate a trend.

According to research, several comorbidities are considered risk factors for mortality and severity in COVID-19 infection. Moreover, a high number of these risk factors further increases this risk. Zheng *et al.* performed a highly credible meta-analysis with a total of 3027 patients. They showed that the proportion of underlying diseases such as diabetes, CVD, and respiratory diseases was statistically higher in critical/mortal patients than non-critical. In addition, underlying comorbidities were factors for disease progression (33).

Therefore, although we could not prove the association, several high-quality studies have observed a significant correlation, and we recommend a larger sample size if our protocol is used for further studies.

Hypothesis 4: the leading cause of death was respiratory complications of COVID-19

Our results showed that an overwhelming percentage of the non-surviving patients died due to respiratory causes. It was challenging to distinguish if they died due to SARS-CoV-2 or due to old age or comorbidities. Therefore, we distinguished between if they died due to respiratory causes compared to non-respiratory causes. In actuality, some categorized as "non-respiratory" may still have died due to the virus, just through another mechanism. However, the reverse is also true; some patients may have died due to non-COVID-19 respiratory causes. Nevertheless, the percentage of the people that died of respiratory causes is still significantly larger than the other group, 81% versus 19%.

This result is substantiated by data provided by the Istituto Superiore di Sanità. They observed ARDS in 96.8% of infected patients dying in the hospital. ARDS also has been proven to increase with increasing age and in subjects with acute heart, liver, and kidney

function disorders (32). Zhou *et al.* remarked that respiratory failure was observed in 98% of those that died (42).

Thus, after examining the studies above, our proportion of those that died of respiratory causes was slightly lower than what is observed in the general population but still constitutes a clear majority.

Hypothesis 5: the time of PCR negativization is longer than in the general population

The fifth hypothesis aims to explore the duration of PCR positivity and if this time is longer compared to the general population. Our study shows a median duration of 19.5 days (IQR 15-27).

The duration of PCR positivity is often used to determine the length of illness and infectiousness. This is not entirely accurate. Various patients have been reported to have prolonged PCR positivity even after symptoms have receded. Some of our patients that were not counted due to incomplete data may be in this category. Furthermore, the presence of viral RNA does not necessarily mean the presence of transmissible virus. Studies have observed that peak infectivity begins just before the emergence of symptoms and drops drastically after the first week of disease. A position statement from the National Centre for Infectious Diseases in Singapore states that viable virus was not detected after the second week of illness despite persistence of PCR detection (59).

Xu *et al.* entered 113 symptomatic participants into their study, yielding a median time of PCR negativization of 17 days (IQR 13-22). In addition, they established that the male gender and severity are independent risk factors for prolonged viral shedding (60). Other studies had a median duration of 20-24 days (61–63). Among the referenced studies, Weiss *et al.* had the narrowest IQR of 16-23 days (63).

Our study with a predominance of female participants (84% N=79) may skew the duration of viral shedding towards a shorter time. Another potential issue is that we included a mix of symptomatic and asymptomatic patients, which may also impact the duration of PCR positivity compared to other studies, especially since higher severity is associated with a prolonged course.

Hence, taking the research into account, we cannot prove any significant difference between our results and theirs. However, more research should be conducted to establish the actual duration of PCR positivity in the general population and among subgroups such as elderly nursing home residents.

Study limitations

The main limitation of this study is the small sample size and uneven gender distribution. There was also difficulty finding appropriate comparative data from the local general population. A suggestion would be to gather the same data from all the patients admitted in the time period and then separate the study population into patients from nursing homes and other patients. Then we could compare the nursing home residents with the general population from the same area admitted within the same timespan. Another study limitation is its retrospective character; the medical records are especially prone to bias. Finally, estimations of viral shedding are limited by the frequency of specimen collection and the RT-PCR tests' sensitivity and specificity.

7. CONCLUSIONS

- The proportion of asymptomatic forms of infection was 16%, this is quite similar to the newer studies' data on the general population, and we could not establish any significant difference. However, there should be more high-quality studies done on this subject.
- We could prove a statistically significant relationship between mortality and increasing age, and this is substantiated by previous research.
- The number of comorbidities was not significantly linked to mortality and severity in our study. Nevertheless, several studies with higher credibility have shown this correlation, leading us to attribute this divergence to our low study population.
- The majority of the patients that died (81%) had respiratory complications and were thought to have died from SARS-CoV-2. Data from the general population also substantiate this.
- The mean duration of PCR positivity in our study was about three weeks. Unfortunately, the research on this topic is limited by huge heterogeneity and should be expanded. Nonetheless, our results were quite similar to the later, more credible articles on the general population.

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9. SUMMARY

Objectives:

The study aimed to explore clinical features and outcomes in elderly nursing home patients.

Materials and methods:

This research was conducted as an observational retrospective study. We included 94 SARS-CoV-2 positive patients admitted to the Hospital of Split from elderly nursing homes from 1 March to 31 May 2020. Medical data were collected by reviewing medical files from the archive at the Clinic of Infectology in University Hospital of Split.

Results:

The ages of the participants range from 47 to 96 years, which we stratified into six age groups, ranging from 45-54 to 95-104. The age-specific mortality rate per 100 was calculated and showed an upward trend after the 65-74 group. This relationship was statistically significant $\chi^2(3, N=93) = 10.21, P=0.037$. After calculating the specific death rate per 100 for the one comorbidity category (24.14) and two or more comorbidities category (29.13), the Chi-square test resulted in $\chi^2(3, N=94) = 0.30, P=0.610$. Furthermore, we could not prove a statistically significant relationship between severity and number of comorbidities with the Chi-square test resulting $\chi^2(3, N=94) = 2.06, P=0.724$. The proportion of symptomatic to asymptomatic patients was 0.84 and 0.16, respectively. 72 patients had relevant information about testing. Respiratory cause of death was the majority with 81%. The range of PCR positivity was 3-56 days with a mean of 21.67, median of 19.5, and interquartile range of 15-27 days.

Conclusions:

Mortality increases with increasing age, and the great majority of the people that died had respiratory complications. The proportion of symptomatic to asymptomatic patients models the recent studies and are 84 and 16%, respectively. We could not prove a statistically significant correlation between the number of comorbidity and severity and mortality although, several studies have shown a link. The mean duration of PCR positivity was 19.5 days, and this is similar to several studies of the general population, although huge heterogeneity is an issue.

10. CROATIAN SUMMARY

Naslov:

Klinička slika i ishod SARS-CoV-2 infekcije u štićenika domova za starije osobe koji su hospitalizirani na Klinici za infektologiju, KBC Split, u razdoblju od 1. ožujka do 31. Svibnja 2020.godine

Ciljevi:

Cilj studije bio je istražiti kliničke značajke i ishode kod starijih pacijenata u staračkim domovima.

Materijali i metode:

Ovo istraživanje provedeno je kao opservacijska retrospektivna studija. Uključili smo 94 pozitivna pacijenta na SARS-CoV-2 primljena u splitsku bolnicu iz domova za starije osobe od 1. ožujka do 31. svibnja 2020. godine. Medicinski podaci prikupljeni su pregledom medicinske dokumentacije iz arhive Klinike za infektologiju splitske bolnice.

Rezultati:

Dob sudionika kreće se od 47 do 96 godina, koje smo stratificirali u šest dobnih skupina, u rasponu od 45-54 do 95-104. Stopa smrtnosti specifična za dob na 100 godina izračunata je i pokazala je uzlazni trend nakon skupine 65-74. Taj je odnos bio statistički značajan $\chi^2(3; N=93) = 10,21, P=0,037$. Nakon izračuna specifične stope smrtnosti na 100 za kategoriju jednog komorbiditeta (24,14) i dvije ili više kategorija komorbiditeta (29,13), Chi-kvadratni test $\chi^2(3, N=94) = 0,30, P=0,610$. Nadalje, nismo mogli dokazati statistički značajnu vezu između ozbiljnosti i broja komorbiditeta s Chi-kvadratnim testom koji je rezultirao $\chi^2(3, N=94) = 2,06, P=0,724$. Udio simptomatskih i asimptomatskih bolesnika bio je 0,84 odnosno 0,16. 72 bolesnika imala su relevantne informacije o testiranju. Respiratorni uzrok smrti bila je većina s 81%. Raspon PCR pozitivnosti bio je 3-56 dana sa srednjom sredinom od 21,67, medijanom od 19,5 i interkvartilnim rasponom od 15-27 dana.

Zaključci:

Smrtnost se povećava s povećanjem dobi, a velika većina ljudi koji su umrli imala je respiratorne komplikacije. Udio simptomatskih i asimptomatskih bolesnika modelira nedavne studije i 84 odnosno 16%. Nismo mogli dokazati statistički značajnu korelaciju između broja komorbiditeta i ozbiljnosti i smrtnosti iako je nekoliko studija pokazalo vezu. Srednje trajanje PCR pozitivnosti bilo je 19,5 dana, a to je slično nekoliko studija opće populacije, iako je problem ogromna heterogenost.

11. CURRICULUM VITAE

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