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MORTALITY OF SARS-COV-2 IN SPLIT, CROATIA

DIPLOMA THESIS

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The doctor of the future will give no medication,
but will interest his patients in the care of the human frame,
diet and in the cause and prevention of disease.
Thomas A. Edison (1847–1931)

I want to express my sincere gratitude to my mentor Prof. Marija Definis, MD, PHD, and the whole Forensics and Pathology Department. It was a pleasure working with you, and I am grateful for this opportunity.
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Abbreviations

ACE2 – angiotensin-converting enzyme 2
ACEi - angiotensin-converting enzyme inhibitor
AMI - acute myocardial infarction
ARDS - acute respiratory distress syndrome
CDC - Centers for Disease Control and Prevention
CFR - case fatality ratio
COVID-19 - corona virus disease 2019
CRP - c-reactive protein
DAD - diffuse alveolar damage
ICU - intensive care unit
IFR - infection fatality rate
MERS-CoV - middle east respiratory syndrome coronavirus
MERS - middle east respiratory syndrome coronavirus
NPI - non-pharmaceutical interventions
OR - odds ratio
PHEIC - public health emergency of international concern
SARS - severe acute respiratory syndrome
SARS-CoV - severe acute respiratory syndrome coronavirus
SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2
STEMI - ST-segment elevation myocardial infarction
UHS - University Hospital of Split
WHO - World Health Organisation

1. INTRODUCTION

For more than a year, many people and the media have been speculating about the mortality of severe acute respiratory coronavirus 2 (SARS-CoV-2). Is the mortality rate higher than that of influenza but lower than that of middle eastern respiratory syndrome (MERS)? Is it equally life-threatening for all age groups?

Ever since its first discovery, SARS-CoV-2 has been a global health concern and has infected a significant part of the world's population (1). Our lives have been impacted by the SARS-CoV-2 pandemic, as well as by non-pharmaceutical interventions (NPI) implemented to reduce transmission rates and the impact of this novel disease. It is known that the majority of NPI's can have a negative impact on the functioning of society, the general well-being of people, as well as the economy. Consequently, they should be utilized to protect the most vulnerable individuals in society, and their introduction based on local epidemiological data (2).

Parameters such as mortality and case fatality play a critical role in planning strategies at national and international levels. When dealing with emerging infectious diseases, however, there can be several factors restricting early accurate mortality estimates (3). In China, initially, a mortality of 15% was estimated, which then dropped to 5.5% (4,5). Through the organized collection of more clinical data, the estimate of the Chinese case fatality ratio (CFR) was set to be 3.5%, or 0.8% excluding Hubei Province, where the outbreak was first noticed (6,7). Health sector decision-makers and crisis management teams are advised to consider a broad range of 0.25%-3.0% for SARS-CoV-2 CFR estimates, as further country-specific studies will be needed to clarify the CFR of this novel disease (6).

1.1 Pandemic Viruses

A pandemic is the worldwide spread of a disease, specific behavior linked to health, or other health-related events crossing international borders and occurring in excess of normal expectancy (8). Previous pandemics and outbreaks with the potential to evolve into such were often caused by viruses (9).

1.1.1 Influenza Viruses

Seasonal influenza, widely known as the flu, is an annually appearing disease characterized by a peak in the winter months. The impact of the disease on public life and the economy is largely manageable, and health care providers are able to keep up with public and patient needs. The mild impact of seasonal influenza on the general functioning of everyday

life is a result of the combined effect of pre-existing immunity from previous exposure and the yearly influenza vaccination (10). Seasonal influenza generally causes a self-limited illness followed by full recovery without requiring any interventions, posing a high risk for serious complications only to certain patient groups. Such high-risk patient groups include infants, elderly, pregnant, obese, and persons with chronic medical conditions. An estimated 70% to 90% of deaths from seasonal influenza occur in people aged 65 years and older (11).

Pandemic influenza on the other hand, is considered uncommon, arising only three times in the 20th century. It possibly poses a high risk for serious complications even to healthy people (10). The CFR of pandemic influenza ranges from 0.03% to 1-3% (11). The majority of people do not have immunity to these new circulating strains, allowing for a much higher impact on the general public, economies, as well as health care system. Consequences can include travel restrictions, school and business closings, and an overwhelmed health system, requiring alternate care sites to meet the increased needs (10). The latest influenza pandemic in 2009 was caused by a novel influenza A (H1N1) virus previously not identified in animals or people, it was named pdm09 (11). Its impact on the global population was significantly less severe than that of previous pandemics with the Centers for Disease Control and Prevention (CDC) estimating 151,700-575,400 people dying worldwide from pdm09 virus infection in the first year the virus spread across the world, with approximately 80% of deaths occurring in people younger than 65 years of age. Since the end of the pandemic, the pdm09 virus continues to circulate as a seasonal influenza virus (11).

1.1.2 Coronaviruses

Coronaviruses are enveloped RNA viruses, their genomes generally encode spike, small membrane, nucleocapsid and, internal proteins. Arrangements of spike proteins embedded in the envelope cause the corona- or crown-like appearance (12). The first human coronavirus was isolated in the year 1965 from the nasal discharge of a patient with a typical common cold (13). Today multiple studies suggest that human coronaviruses are second only to the family of rhinoviruses as causes of the common cold (14). In the 21st century, multiple novel coronavirus strains have emerged and caused severe respiratory disease in humans (7,15,16).

1.1.2.1 Severe Acute Respiratory Syndrome Coronavirus

In the early months of 2003, outbreaks of severe acute respiratory syndrome (SARS), atypical pneumonia caused by an unknown airborne infectious pathogen emerged in the Guandong province of China. Within little time it spread to cause clusters in multiple cities around Guangzhou. By May 2003, a novel coronavirus was identified as the causative pathogen of the pandemic (15,17). The severe acute reparatory syndrome coronavirus (SARS-CoV) spread to 30 countries, severely becoming embedded in six of them. It infected 8439 people and caused 813 fatalities in its course, resulting in a CFR of 9.6%, eventually declared as contained on the 5th of July 2003 by the World Health Organisation (WHO) (18).

The SARS pandemic began in an unusual pattern. Index cases were commonly identified among healthcare workers, making up approximately 20% of all cases. The virus spread further to family members and other close contacts. From there, the virus spread into the larger communities (18,19). The median incubation time averaged 6 days, followed by the onset of high fever as the most common symptom. Other symptoms include chills, myalgia, cough, headache, and dizziness (19). Pneumonia can develop, which in severe cases then progresses to acute respiratory failure, requiring mechanical ventilatory support. Hematologic findings are thrombocytopenia, lymphopenia, prolonged activated partial thromboplastin time, and elevated D-dimer levels. Significant predictive factors of intensive care unit (ICU) admission and death are advanced age and male sex (19).

Macroscopic examination of the lungs shows gross consolidation. Histopathological examination of lung tissue sampling displays diffuse alveolar damage (DAD) at multiple stages of progression and severity. The early phase is characterized by pulmonary edema with hyaline membrane formation, while fibromyxoid organizing exudates indicate the organizing phase of DAD (19). Further common pathologic hallmarks are desquamation of pneumocytes in alveolar spaces, interstitial mononuclear infiltrates, necrotic inflammatory debris, and focal intra-alveolar hemorrhage (15). Viral inclusions and other organ involvement are not evident (19). It is suggested that severe and fatal SARS-CoV cases are a result of secondary vascular and inflammatory disease caused by immune response dysregulation and variable host factors, rather than due to the combined occurrence of bacterial and viral pneumonia, as is the case of influenza (20).

1.1.2.2 Middle East Respiratory Syndrome Coronavirus

In June 2012, several years following the SARS pandemic, another previously unknown coronavirus was detected. It was first isolated from a patient who died from progressive respiratory and renal failure in a hospital in Jeddah, Saudi Arabia (16,18). From here on, cases of MERS have been reported in 27 countries. Presenting symptoms range from none to mild or severe respiratory complaints, fever, cough, and dyspnea (21). These symptoms can be accompanied by gastrointestinal symptoms, lymphopenia, neutrophilia, and elevated liver enzymes later in the course of the disease (16,21). Susceptible patients are at an elevated risk of more severe disease, possibly leading to respiratory failure, requiring mechanical ventilation and ICU support. Such patients commonly include the elderly, immunocompromised, and patients with chronic diseases, as for example cancer, chronic lung disease, diabetes, or renal disease (21).

Middle east respiratory syndrome coronavirus (MERS-CoV) was isolated in dromedaries in several countries in South Asia, Africa, and the Middle East. It is believed to have originated in bats and then have spread to camels at some point in the past. Infection occurs through direct or indirect contact with infected dromedary camels, person to person spread among infected people in health care settings, and rarely through close contact such as among family members (21). The median age of patients infected with MERS-CoV is 51-57 years, with a male predominance. No significant differences were observed in the comparison of demographic and epidemiological characteristics of cases in the period from 2015 to 2020 (22).

Despite a large number of infected and deceased patients with MERS, it was years after the emergence of the virus when Ng *et al.* were the first to report an autopsy of a MERS case (23). Massive pleural, significant pericardial and abdominal effusion, as well as generalized congestion, were present. Major histologic pulmonary findings consisted of exudative phase DAD with denuded bronchiolar epithelium, prominent hyaline membranes, and alveolar fibrin deposits. Furthermore, hyperplasia of type 2 pneumocytes, rare multinucleated syncytial cells, and intra-alveolar, as well as subpleural foci of necrotic debris were found (23). The alveolar septa were edematous and infiltrated by lymphocytes with few plasma cells, neutrophils, and macrophages. Viral inclusions were not seen. Tracheal and bronchial sections showed lymphocytic mucosal and submucosal inflammation, with plasma cells and few neutrophils (23).

The MERS virus was never contained, and outbreaks still emerge occasionally. As of January 2021, a total of 2566 laboratory-confirmed cases have been reported worldwide. This number includes 882 fatal cases, resulting in a CFR of 34.4% (22). The WHO remarks that this mortality may be an overestimate due to missed mild cases, as only laboratory-confirmed cases are being counted (21).

1.1.2.3 Severe Acute Respiratory Syndrome Coronavirus-2

The Coronavirus disease 2019 (COVID-19) is a respiratory syndrome caused by SARS-CoV-2 (24). Early research proved it to be a novel coronavirus showing high sequence identity with a lineage of SARS-like coronaviruses present in bats (7).

1.1.2.3.1 Epidemiology

In late December 2019 in Wuhan, Hubei province in China increased presentation of patients with pneumonia of unknown origin was observed. The majority of patients were connected to a local food market in Wuhan, Hubei Province in China (7). Newer studies proved retrogradely that SARS-CoV-2 had been spreading around the globe by that time already (25,26).

Out of the early cases, 55% were linked to the Huanan Seafood Wholesale Market. This number decreased in late December to only 8.6%, with an exponential increase in the number of nonlinked cases, resulting from community spread within new clusters outside of the market. The mean incubation period from infection to illness onset is 5.2 days, while its 95th percentile of distribution is 12.5 days, suggesting a 14-day quarantine period to monitor and screen among exposed patients (27). With an estimated basic reproductive number R_0 of 2.2 (95% CI, 1.4 to 3.9), the number of infected doubled in size every 7.4 days. Early on, it was concluded that human-to-human transmission occurred among close contacts, necessitating the implementation of measures to prevent further transmission (27). While there have been studies showing the strong survival ability of SARS-CoV-2 in human specimens and on surfaces in the environment for multiple days, none of them are representative of real-life situations, leaving the expected chance of transmission through inanimate surfaces very small (28,29).

The all-cause mortality (Figure 1), as well as the mortality of pneumonia-specific deaths (Figure 2) in Wuhan, registered a steep increase in the fourth week of 2020. Taking the median time delay of 17 days from disease onset into account, this implies that SARS-CoV-2 was already widely prevalent among the population of Wuhan by the first week of 2020. However,

the absence of unexpected fluctuations before December 2019 does not exclude a possible low-level circulation of SARS-CoV-2, as mortality changes at the population level would be unlikely to be sensitive enough to detect this (30).

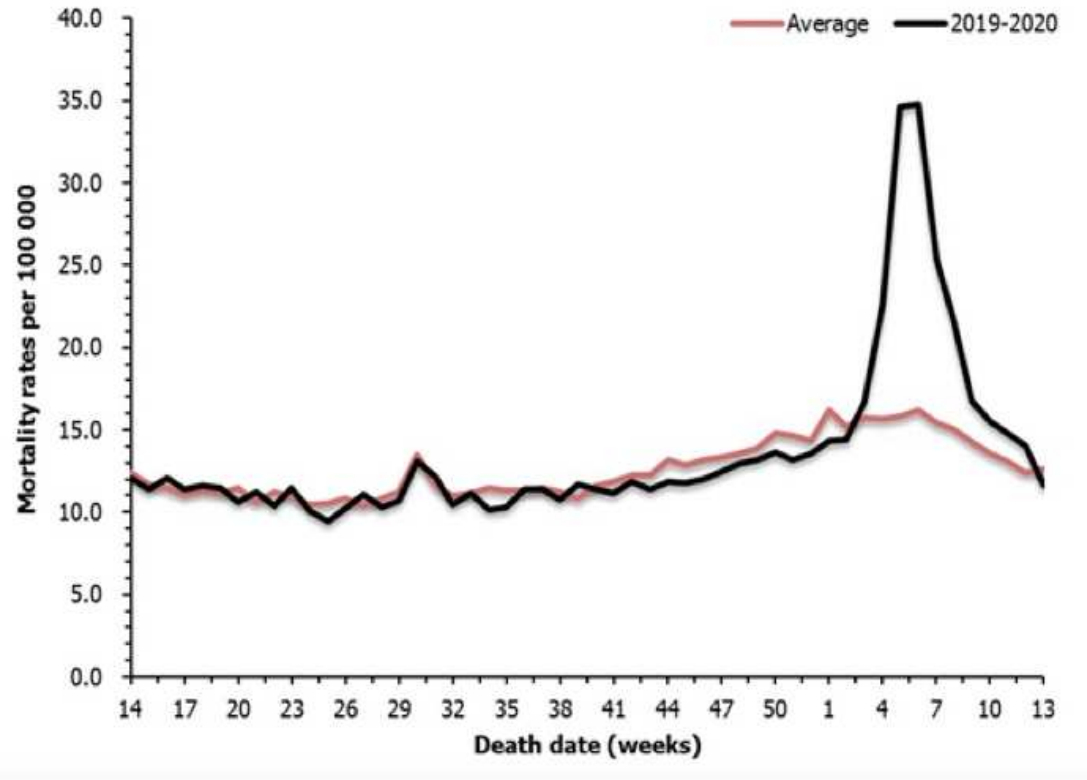


Figure 1. Comparison of trends of the all-cause mortality rate in 2019-2020 against the average rate of 2016-2018 in Wuhan, for all age groups. Source: WHO-convened Global Study of Origins of SARS-CoV-2: China Part (30).

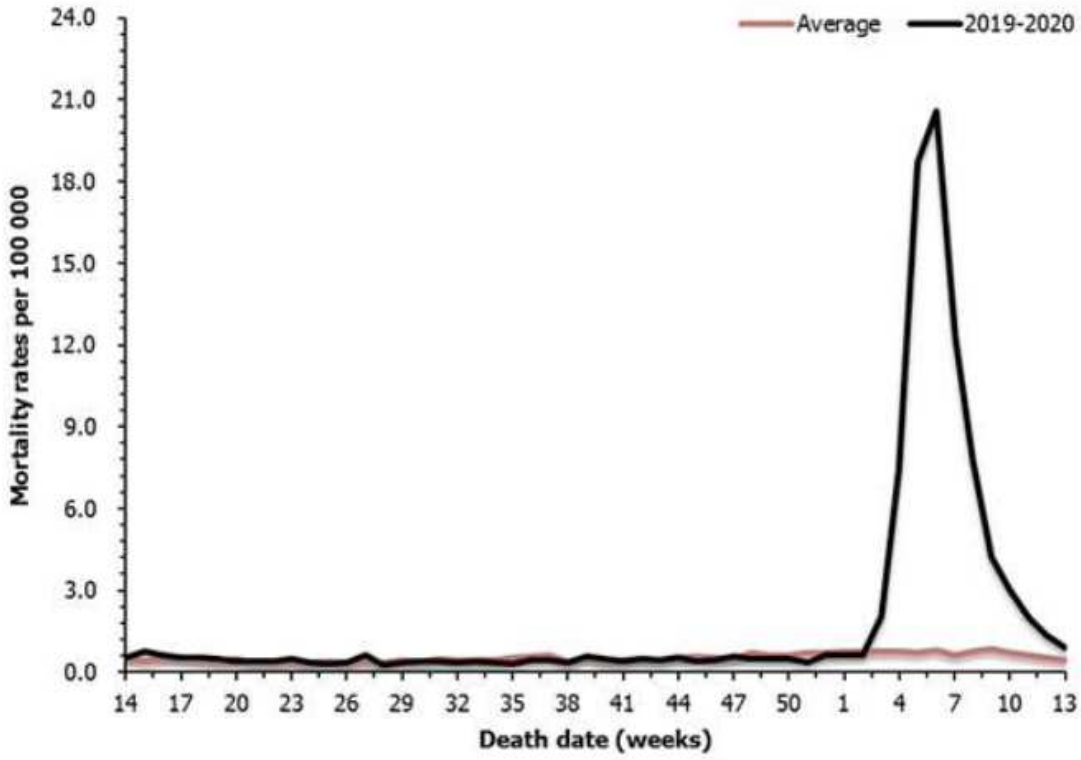


Figure 2. Comparison of trends of the pneumonia mortality rate in 2019-2020 against the average rate for 2016-2018, Wuhan, for all age groups. Source: WHO-convened Global Study of Origins of SARS-CoV-2: China Part (30).

By the end of January 2020, there were 98 recorded cases in 18 countries outside of China, and the WHO declared this novel coronavirus outbreak a Public Health Emergency of International Concern (PHEIC) (1). In March, worried by the concerning levels of global inaction following warnings and guidelines, as well as the extent and severity of spread, the WHO declared the situation a pandemic, and Europe had become the new epicenter of it (1).

One year after the discovery of the global spread of SARS-CoV-2 the WHO dispatched an international expert team to China to evaluate four scenarios regarding the introduction of this novel coronavirus, as seen in Figure 3. In decreasing order of likelihood, according to the WHO expert team:

- introduction through an intermediate host, then followed by spillover to humans
- direct zoonotic transmission (spillover)
- introduction through the cold/food chain
- introduction through a laboratory incident (30).

Although the team could not exclude any of the proposed scenarios, they estimated the relative likelihood of the pathways based on their qualitative risk assessment. Introduction through an intermediate host was considered the most likely, followed by direct zoonotic transmission and introduction through the cold/food chain, whereas introduction from a laboratory incident was considered to be extremely unlikely (30).

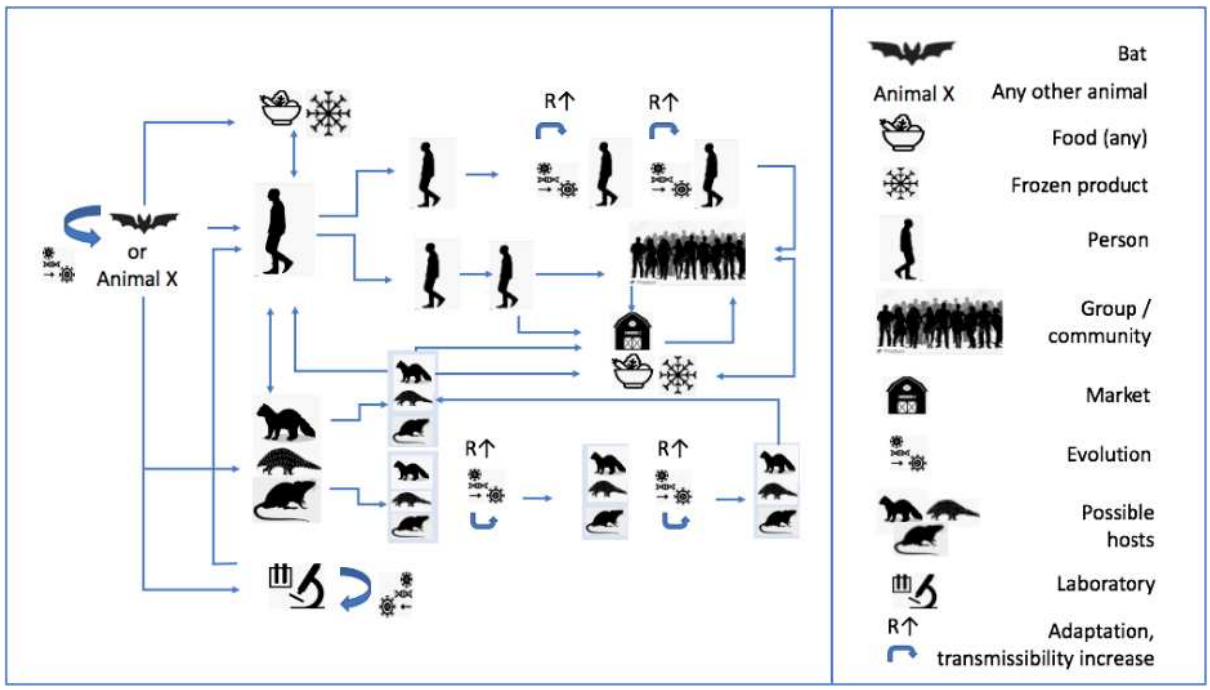


Figure 3. Overall schema for possible pathways of emergence, providing a conceptual framework for possible routes for SARS-CoV-2 emergence. The icons are meant to be interpreted in a generic manner and the location and timing is not stated. The animals depicted reflect animal species that have been discussed in relation to potential infection but can be replaced by other species as well. Arrows indicate directions of possible transmission. The symbols indicating "evolution" are meant to reflect any mutations, recombination, variant selection leading to enhanced ability to infect other species and/or transmit. Source: WHO-convened Global Study of Origins of SARS-CoV-2: China Part (30).

While the early reports described the initial cases of SARS-CoV-2 in late December 2019 in China, Deslandes *et al.* were the first to report a retrogradely identified SARS-CoV-2 case in that period in Europe (7,26). The patient was identified by reverse transcription-polymerase chain reaction analysis of frozen respiratory samples after he presented with a four-day history of hemoptysis, cough, chest pain, headache, and fever on 27 December 2019 in a hospital near Paris, France (26). The patient neither had a link to China nor any recent travel

history, but his child reported an influenza-like illness prior to his onset of symptoms, suggesting that the virus was already circulating in France by the end of December 2019 (26).

An Italian group of researchers found SARS-CoV-2 receptor binding domain-specific antibodies in 111 out of 959 samples (prevalence of 11.6%) of a prospective lung cancer screening trial covering September 2019 until March 2020, including all regions in Italy. They identified the first positive sample on 3 September 2019, and by the end of September positive samples were found in eight out of 20 regions (25). This suggests that SARS-CoV-2 circulated in Italy months before the initial reports from the Wuhan clusters, allowing for underestimation of the overall COVID-19 cases (7,25). All patients were asymptomatic at the time the samples were collected (25).

1.1.2.3.2 Symptoms

The spectrum of clinical signs of COVID-19 is heterogeneous, ranging from mild influenza-like symptoms in most cases to severe and live endangering complications such as acute respiratory distress syndrome, organ failure, or shock that can ultimately result in death (4,31). Characteristic clinical signs observed to occur with high prevalence are olfactory and gustatory dysfunctions, such as anosmia, hyposmia, ageusia, and hypogeusia. Increased awareness of this fact and screening for such conditions could contribute to improved identification of patients with COVID-19 (32). Most frequently, an infection with SARS- CoV-2 presents with fever, cough, fatigue, and myalgia, in order of decreasing frequency. Further symptoms include anorexia, chest tightness, dyspnea, and muscle soreness. Less commonly, headache, pharyngalgia, gastrointestinal symptoms, and shivering appear. A small percentage of patients do not develop symptoms at all (5). Complications commonly observed in patients with fatal disease outcomes include ARDS, sepsis, cardiac injury, and respiratory failure (33).

Common shifts of laboratory values during an infection with SARS-CoV-2 are elevated c-reactive protein (CRP), erythrocyte sedimentation rate (ESR) levels, and normal leukocyte counts with lymphopenia (5). Levels of D-dimers greater than 2.0µg/mL on admission are proven to be an early effective predictor of in-hospital mortality, allowing for better management of severe COVID-19 patients (34). In fatal cases, elevated levels of lactate dehydrogenase, D-dimer, CRP, and sometimes mild thrombocytopenia are seen (35).

The majority of patients have bilateral lung lesions on chest x-ray, while approximately 25% have lesions involving only a single lung (5). On chest computed tomography, the characteristic ground-glass opacifications with subsegmental signs of consolidation are visible.

In ICU patients, typically, there are extensive subsegmental and lobular areas of consolidation bilaterally (4).

1.1.2.3.3 Pathologic findings

SARS-CoV-2 is known to most commonly cause respiratory disease, but it also poses a risk of causing systemic inflammation (33). Vascular complications are one of the key dangers in COVID-19, next to respiratory disease. This is reflected in autopsy findings, identifying venous thromboembolisms in a large proportion of COVID-19 patients, even though it has not been suspected in these cases before their death. In all patients identified with deep venous thrombosis in the legs, they tended to occur bilaterally. Another common vascular complication in COVID-19 patients are pulmonary embolisms. In a large part of patients such an occurring pulmonary embolism originating from the lower extremity deep veins will be the direct cause of death (35). A recent meta-analysis also found an increased risk of ischemic and cryptogenic stroke with an odds ratio (OR) of 3.58 and 3.98 respectively, in patients with SARS-CoV-2 infection, possibly increasing the mortality risk (36). The increased incidence of such thromboembolic events in patients with COVID-19 suggests it having an impact on the coagulation system (35).

Post-mortem computed tomography of the lungs shows various patterns of reticular infiltrations bilaterally, with dense, severe, consolidating infiltrates. In COVID-19 patients, such findings will be present in the absence of any pre-existing lung pathologies like a tumor or emphysema (35). Typically, the lungs are heavy and congested, weighing up to three times as much as a physiological lung. The lung surface is commonly marked with mild pleurisy and a mosaic-like pattern which is made up of pale areas and firm slightly protruding, dark purple hypercapillarized sections. This pattern is likewise present at all cut surfaces (35,37). At times, changes in the lungs appeared as respiratory tract infections of the purulent typed with abscessed bronchopneumonia. In such cases, the characteristic macroscopic signs of ARDS were absent or only mildly visible (37). No macroscopical changes are evident outside the respiratory tract and lungs except for splenomegaly in some cases, suggesting viral infection. High amounts of viral RNA were consistently found in the lungs of patients infected with SARS-CoV-2 but at times also in systemic circulation as viremia, in the liver, kidney, and heart tissue. A positive correlation between morbidity and obesity was observed by Wichmann *et al.*, reporting a mean body mass index of 28.7 kg/m^2 among fatal cases (35).

Histopathological examination of the lung tissue shows DAD consistent with the early phase of ARDS, with capillary congestion, hyaline membranes, protein-rich exudate, fibroblasts, and activated type II pneumocytes. Advanced stages are characterized by fibrosis and squamous metaplasia, in some cases with megakaryocytes and giant cells. In cases of exacerbated purulent bronchitis, focal confluent bronchopneumonia can dominate the tissue findings or give rise to mixed states of purulent pneumonia and DAD in different stages of organization (35,37). Microthrombi are commonly found in the smaller arteries of the lungs, at times also within the prostate, but never in other organs (35). Examination of lung tissue from patients with COVID-19 with electron microscopy shows characteristic vascular pathobiological features such as ultrastructural endothelial damage and distorted vascularity with structurally deformed capillaries. Furthermore, elongated capillaries, intracapillary intussusceptive pillars, and changes in caliber, as well as intra and extracellular presence of SARS-CoV-2 are visible (38). Pathological characteristics such as destruction of alveolar septae, bronchial lymphocytic infiltration, and extrapulmonary findings like myocardial hypertrophy or scarring can be apparent in patients with pre-existing conditions. Extrapulmonary signs confluent with a viral infection like lymphocytic pharyngitis presenting with hyperemic mucosa can be found in almost all patients (35,37).

1.1.2.3.4 Mortality

When dealing with novel pathogens, it is of great importance to know the severity of the disease, its ability to cause death. Fatality rates allow us to quantify the graveness of a disease, identify populations at risk and assess the quality of healthcare in times of increased burden (39). Two parameters are commonly used to assess the fraction of infected individuals experiencing fatal outcomes, infection fatality ratio (IFR) and CFR. IFR evaluates the proportion of fatalities among all infected individuals, whereas CFR estimates the proportion of death only among confirmed identified cases. Hence, at this early stage of the pandemic, fatality rates are expressed in CFR (39). The reported mortality for COVID-19 has been following a trend that is typical for emerging infectious diseases, with a decrease of CFR in the initial phase of an outbreak (3). First reports from China described a CFR of 15%, but with more data being published, the CFR quickly decreased to 5.5% (4,5). It has to be noted that multiple factors can restrict acquiring a precise CFR estimate. Health care capacity, resources, and preparation can alter outcomes notably. Singapore implemented widely available testing, proactive contact tracing, and containment measures, keeping their CFR as low as 0.05%. In

other countries, such measures might not be available, resulting in a smaller denominator and skewing to a higher CFR (3). Therefore, an accurate denominator is of uttermost importance for the accurate calculation of the CFR. Patients exhibiting mild or no symptoms, misdiagnosed, and untested persons could be overlooked, leading to an overestimation of the CFR (as the WHO suggests might be the case for the CFR of MERS) (3,21).

With the SARS-CoV-2 outbreak on board the Diamond Princess cruise ship, a unique setting for a quite accurate estimate of the CFR arose. The passengers were quarantined onboard of the ship from 20 January to 29 February 2020. This situation provided a population located in a defined territory on board the ship, without the majority of usually occurring confounding factors, such as lack of testing or imported cases. There were 3711 people on the ship, out of which 705 contracted the disease and tested positive for COVID-19, while seven died. This results in a CFR of 0.99% (3).

As mentioned earlier, the accuracy of the CFR depends on different factors, which is further complicated with different countries each having individual strategies to test and report COVID-19 fatalities, making comparisons challenging (3,40). It is due to these facts that various countries started using excess mortality as simple means to be able to compare mortality. Excess mortality is defined as the difference in the total number of deaths during a crisis set against the expected mortality under usual conditions (40). On 30 January 2020, when the WHO declared the SARS-CoV-2 outbreak a PHEIC, the official COVID-19 death toll was 171. Almost one year later, by 31 December 2020, this number increased to 1.813.188. The WHO suggests an excess mortality of at least 3.000.000 for that period (40).

Recent studies suggest that a big part of excess death is likely caused by undiagnosed COVID-19. A smaller but principal part of excess mortality occurring outside of hospitals though stems mainly from cancer and cardiac disease, suggesting patients avoid hospital care during this ongoing pandemic (41). Vrdoljak *et al.* assessed the effects of COVID-19 anti-epidemic measures in Croatia and their successive effects on the function of the health care system. After the introduction of the first lockdown in Croatia, the number of newly diagnosed breast cancer cases decreased by 24% during the months April, May, and June 2020 in comparison to the same months in 2019. This is likely to result in a later diagnosis, initiation of treatment, and less favorable outcomes in the future (42).

Similarly, during periods of lockdown hospital admissions decreased for all non-COVID-19 disease groups compared to prepandemic baseline periods. Additionally, mortality rates were increased during and in-between lockdowns for patients admitted with cancer,

sepsis, respiratory disease, and pneumonia (43). This is reflected in an Italian study, showing a 48.4% reduction in admissions for acute myocardial infarction (AMI) compared to an equivalent week in prepandemic times. At the same time, a substantial increase of ST-segment elevation myocardial infarction (STEMI) CFR with an increased risk ratio of 3.3 was observed (44).

These insights suggest that fear of being infected by SARS-CoV-2 in a hospital setting may cause patients to stay at home instead of going to the hospital. When they do seek medical help, it is often late in the course of the disease, and their condition is already crucial. Patients avoiding or delaying care for life-threatening conditions due to fear of the pandemic may add an increased mortality rate of treatable diseases on top of the one that is directly associated with COVID-19 (45).

1.1.2.3.5 Risk Factors

High age and comorbidities such as diabetes and cardiovascular or other chronic illnesses are shown to be significant factors affecting morbidity and mortality in patients with COVID-19, greatly influencing the prognosis of the disease course (33,46,47).

1.1.2.3.5.1 Age

Older age is considered a high-risk factor for developing serious complications of COVID-19. An increased need for hospitalization after infection with SARS-CoV-2 has been reported in multiple studies in the elderly, just as there was a significantly increased risk of death from COVID-19 in patients ≥65 years old (OR 4.59) (47,48). While the age-specific CFR in young is considered negligible, it rises above 40% at age 95 years (49). Looking at the possible complications of a SARS-CoV-2 infection, there is a linear relationship between patient age and the incidence of ARDS in patients with COVID-19. With every one-year increase of patient mean age, the incidence of developing ARDS rises statistically significantly by the factor 2.9. Besides the development of ARDS, a significant linear relationship between patient age and the development of acute cardiac ischemia as well as acute kidney injury has been discovered. Incidence rates in the elderly increase by a factor of 1.6 and 0.4 respectively for every mean age increase of one year (50).

Dhar Chowdhury *et al.* even propose that the remarkable difference of the overall CFR between Italy (7.2%) and China (2.3%) in the early phase of the pandemic was likely due to the higher rate of infections among the elderly population in Italy. The proportion of cases in

persons older than 70 years was 37% in Italy vs. 11% in China. While the age-specific CFR in the age groups <70 years of age were similar between China and Italy (8% and 12.8%, respectively), it was significantly higher in the Italian population >70 years (20.2% and 14.8% respectively) than in the Chinese (51).

3.1.2.3.5.2 Comorbidities

Commonly occurring comorbidities in patients with severe or fatal COVID-19 are obesity, hypertension, diabetes mellitus, and cardiovascular disease, in order of decreasing prevalence. Less common comorbidities are respiratory and cerebrovascular disease, malignancy, chronic kidney, and liver disease. Previous respiratory disease is relatively uncommon among patients with COVID-19, but it is by far the most strongly predictive comorbidity for adverse outcomes associated with it (52). Some of the first papers reporting autopsies of COVID-19 cases reported pre-existing chronic medical conditions in all but a few cases (52).

Obesity, defined as a body mass index of over 30 kg/m^2, is the most prevalent comorbidity in patients with severe or fatal COVID-19 and significantly elevates the risk of requiring invasive mechanical ventilation during the disease course (52).

In patients with diabetes, invasive mechanical ventilation and ICU care are more likely to be required upon infection with SARS-CoV-2. Furthermore, they have an increased incidence of development of acute kidney injury, compared to COVID-19 patients without diabetes (48). Diabetes is also directly correlated to the mortality of patients with COVID-19, increasing the risk of a fatal outcome in affected patients by the factor 1.75 (53). Underlying mechanisms being discussed as the link of diabetes and COVID-19 are chronic inflammation, increased coagulation activity, potential direct pancreatic damage, and immune response impairment. But it is still unknown whether hyper- and hypoglycemia alter the virulence of SARS-CoV-2, and how inflammatory and immune responses come to be in this group of patients, in regard to their diabetic disease (54).

Hypertension causes an almost 2.5-fold increase of risk of severe COVID-19 disease course, and a 2.4-fold increased mortality risk, especially in patients over the age of 60 years (55). Severely affected patients requiring treatment in the ICU in turn, have a 13-fold increased incidence of acute cardiac injury compared to non-ICU patients (46). In addition, mortality rates are higher in hypertensive patients taking an angiotensin-converting enzyme inhibitor

(ACEi) or angiotensin II receptor blocker compared to those not taking either, with the highest increase of mortality in patients taking ACEi's (48).

Smoking is another proven risk factor for severe, possibly lethal COVID-19 disease course. Patanavanich *et al.* reported patients with a history of smoking to have 1.91 times the odds of disease progression compared to patients who never smoked. Due to the limitations in the analyzed papers, the authors comment that the actual risk of smoking may be even higher (56).

3.1.2.3.5.3 Gender

Among reports of hospitalized patients infected with SARS-CoV-2 as well as among fatal cases, a male predominance can be observed (5,35,48). This is reiterated by the results of an early Wuhan study, where 73% of deceased were male versus 55% of recovered patients (33). Further, coagulopathic events such as deep venous thrombosis and pulmonary artery embolisms, the latter often fulminant and fatal in course, tend to occur in COVID-19 fatalities with a male to female ratio of 1.9:1 (37).

Recently a potential sexual dimorphism in the expression of angiotensin-converting enzyme 2 (ACE2) is being discussed, as it has a key role in cell entry of SARS-CoV-2. However, sex-dependent differences in ACE2 expression have yet to be proven by preclinical evidence. Possible further explanations for worse outcomes in male patients with COVID-19 are a variation of responses to viral infections between the two sexes and distinct immune and inflammatory statuses linked to comorbidities like obesity, hypertension, or age (57).

2. OBJECTIVES

2.1. Aims of this study

The aim of this study was to investigate the mortality of COVID-19 in Split, Croatia, compare the number of hospital deaths in the period of the 7th of April 2020 until the 7th of April 2021 to those of previous years, and identify a possible skewed age pattern among fatal cases infected with SARS-CoV-2. An additional goal was to estimate a first crude hospital mortality for the University Hospital of Split (UHS) and a CFR for the Split-Dalmatia-county.

2.2. Hypotheses

1. In the first year after the earliest fatal case of a SARS-CoV-2 infection, there was an increased hospital mortality.
2. Over 80% of all fatalities of COVID-19 are ≥65 years old.

3. MATERIALS AND METHODS

3.1 Study Design and Data collection

The study was designed as an observational cross-sectional study. Data was collected for the period from the first COVID-19 death in Split, Croatia on the 7th of April 2020, until one year later, the 7th of April 2021. Data was obtained by reviewing the coroner logbooks of the cemetery Lovrinac, the UHS, and the city of Split coroners, as well as the patient database of the Department for Pathology, Forensic Medicine and Cytology of the UHS. Collected data includes age, gender, date of death, SARS-CoV-2, and hospitalization status preceding death. Inclusion criteria are SARS-CoV-2 positive status and death in the city of Split (in- and outside the UHS). Exclusion criteria were death occurring outside of Split and incomplete data.

Further data for calculation of mortality and CFR was obtained from the government website containing raw data COVID-19 pandemic collection, as well as from the UHS (58).

The study was approved by the Ethical Review Committee of the UHS on 24 June 2021 (class: 500-03/21-01/125, number: 2181-147/01/06/M.S.-21-02).

3.3 Study population

A total of 2222 patients were included in the study, out of which 1333 were male and 889 were female. We excluded 10 patients who died outside and one who died inside the hospital, all due to an unconfirmed (suspected positive) COVID-19-status. Further excluded patients were 10 out-of-hospital cases due to incomplete data and an additional three fatalities out-of-hospital fatalities as they occurred outside of the city coroner jurisdiction.

3.4 Data analysis

All statistical analyses were performed using JASP software v. 0.13.1.0 (JASP Team, 2018, Amsterdam, Netherlands).

For calculation of mortality and CFR values, we used formulas provided by the CDC (59). The Single Sample t-Test was used to compare continuous variables with a sample size smaller than five results were considered significant if $T_{stat} < T_{crit}$ (60).

Descriptive statistics for non-continuous variables were expressed using frequencies and percentages. For the descriptive statistics of continuous variables, the Shapiro-Wilk test was first used to determine data distribution for all continuous variables. Data that was not normally distributed were presented using median and interquartile range (IQR). The Mann-Whitney-U test was used to compare continuous variables with a non-normal distribution and

the χ^2 test for comparison of non-continuous variables between groups. A *P*-value with a significance cut-off of $P<0.05$ was used.

4. RESULTS

We collected data on 2222 participants. 572 deaths were attributed to COVID-19 and occurred in the in-hospital setting. An additional 36 cases of COVID-19 positive outpatient deaths and 1614 cases of non-COVID-19 deaths were reported in the hospital (Table 1).

For COVID-19 cases that were in the hospital, there was a male predominance (N=363, 63.29%) and fewer female patients (N=210, 36.71%). Outpatient COVID-19 cases had a male predominance (N=22, 61.11%), with fewer females (N=14, 38.39%). Non-COVID-19 hospital cases had a male predominance as well (N=949, 58.80%), with fewer females (N=664, 41.14%).

The median age at death for hospital COVID-19 cases was 78.5 years (IQR=70.0-85.0), while non-COVID-19 fatal hospital cases had a median age of 77.0 years (IQR=66.25-84.0). Using the Mann-Whitney-U test, we determined that there was a significant difference between the age of death for these two groups (P=0.001), with a higher age at death for hospital COVID-19 cases. The median age at death for outpatient COVID-19 cases was 84.5 years (IQR=72.0-89.0). There was no significant difference in median age at death for this group compared to hospital COVID-19 cases (P=0.051). Using the χ^2-test, we found no gender differences between COVID-19 and non-COVID-19 hospital cases (χ^2=3.49, P=0.059), nor between COVID-19 hospital and outpatient cases (χ^2 =0.07, P=0.793).

Table 1. Characteristics of study participants

	COVID-19 positive out-of-hospital death (N=36)	COVID-19 positive hospital death (N=572)	Non-COVID-19 hospital death (N=1614)
Men	22 (61.11)	362 (63.29)	949 (58.80)
Women	14 (38.39)	210 (36.71)	665 (41.20)
Median age at death in years	84.5 (72.0-89.0)	78.5 (70.0-85.0)	77.0 (66.25-84.0)

Data presented as whole number (%) or median (IQR)

Figure 4 shows the distribution of the three groups of patients according to their age group in steps of 10 years. All three groups had their peaks of fatal cases in the age group 80-89 years. When looking at ages lower than 40 years, non-COVID-19 hospital deaths include a smaller peak in the age group 0-9 years. COVID-19 positive out of hospital deaths exclusively occurred in patients over the age of 50 years, with the highest incidence in the group 80-89 years.

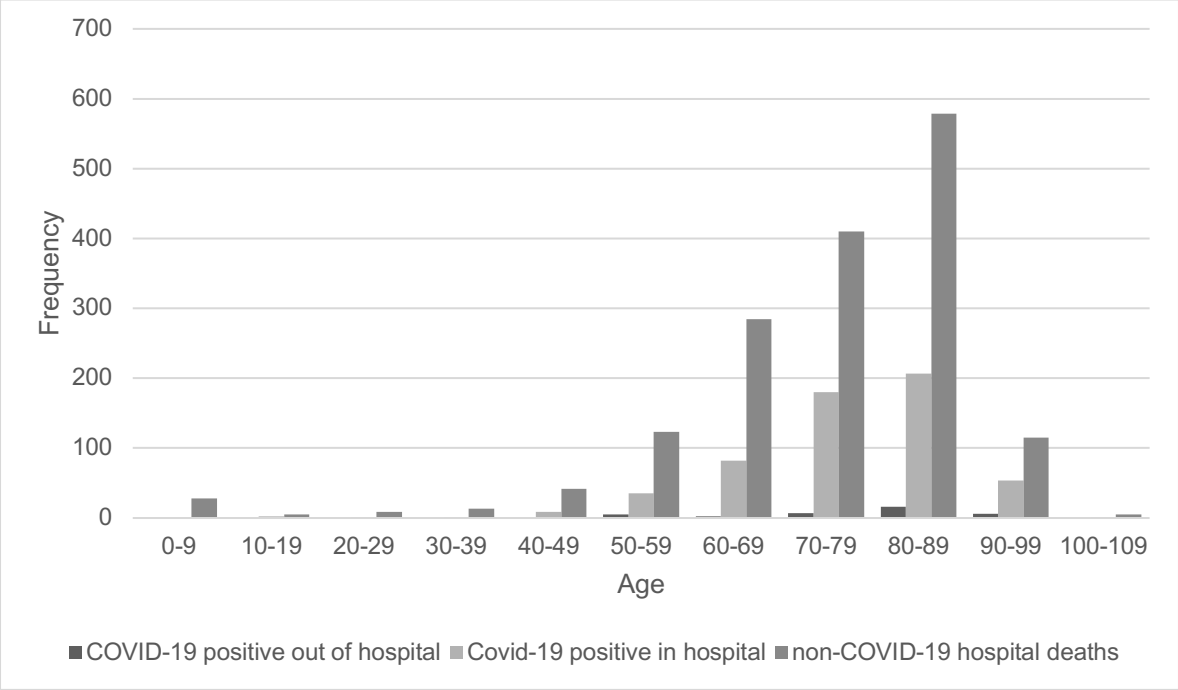


Figure 4. Age distribution of deceased

In a timely distribution of hospitalized COVID-19 fatal cases, we can see two mortality waves. The first one peaking in week 18, the second one in week 50 in 2020, even surpassing the number of general hospital death cases, while there has been a comparably stable distribution of non-COVID-19 hospital deaths with minor fluctuations and peaks (Table 6).

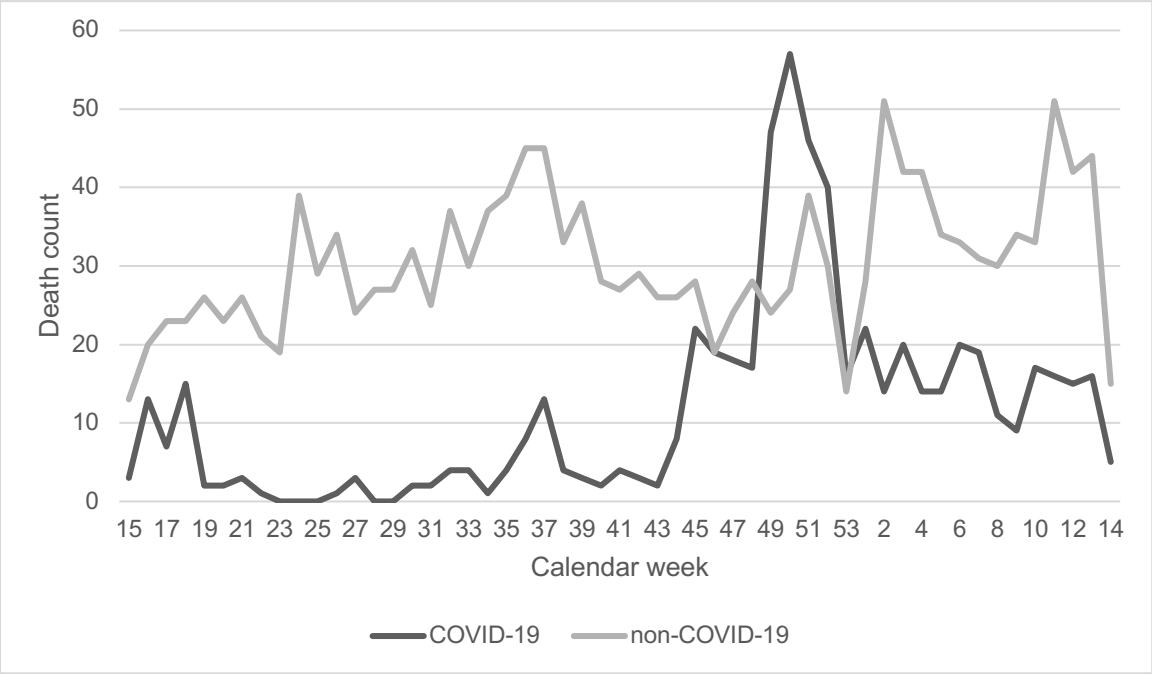


Figure 6. Frequency of hospital deaths per calendar weeks

There has been an increasing downward trend of hospital deaths in the presented time, the mean difference in the period 2016-2020 was a yearly decrease of 124.33. The number of hospital fatalities has then peaked at 2186 in the one year from the first fatal COVID-19 case in Split, Croatia. While this is an increase of 449 compared to the year 2019/2020, it is only an increase of 76 cases if compared with 2016/2017 (Table 2).

To check whether there was a significant increase of hospital deaths in the period 2020/2021 compared to the mean number of hospital deaths of the previous four years, we used the Single Sample t-Test. First, we defined the null hypothesis H_0 $\mu=2186$ and the alternative hypothesis H_1: $\mu\neq2186$. We calculated the sample variants $S_2=25978.91$ and standard deviation for our sample $S=161.18$. We then used the formula for calculation of the Single Sample t-Test $t=\frac{M-\mu}{s/\sqrt{n}}$ to fill in our variables $t=\frac{1938,75-2186}{161.18/\sqrt{4}}$. Lastly, we compared our $T_{stat}=-3.07$ with the $T_{crit}=3.18$. Since $T_{stat}<T_{crit}$ we can reject the H_0 hypothesis and conclude that the two values are significantly different. There was a statistically significant increase of hospital deaths in the period 2020/2021, compared to the mean of the four previous yearly periods.

Table 2. Number of hospital deaths from the 7th of April 2016 until the 7th of April 2021 divided in five yearly periods

Time period	Number of deaths in the UHS	Difference to previous yearly period
07.04.2020-07.04.2021	2186	+449
07.04.2019-07.04.2020	1737	-156
07.04.2018-07.04.2019	1893	-122
07.04.2017-07.04.2018	2015	-95
07.04.2016-07.04.2017	2110	
Mean of period 2016-2020	1938,75	124.33

We used the formula $\text{CFR}=\frac{\text{Number of cause-specific deaths among the incident cases}}{\text{Total number of incident cases}} \times 10^{n}$ to calculate a crude CFR for SARS-CoV-2 in the Split-Dalmatia-county. After adding our values $\text{CFR}=\frac{608}{37497}\times 100$ we got our crude $\text{CFR}=1.62\%$.

To calculate a crude hospital mortality rate for COVID-19 patients in the UHS we used the formula $\text{COVID-19 hospital mortality}=\frac{\text{COVID-19 hospital deaths}}{\text{COVID-19 hospitalizations}}\times 100$. After adding our data $\text{mortality rate}=\frac{572}{2643}\times 100$ we received a crude hospital mortality of 21.64%.

5. DISCUSSION

This study looked into the fatal cases of COVID-19 in the second-largest city of Croatia, Split. Our results demonstrated that there was an increased overall mortality of patients in the UHS in the one-year period from the 7th of April 2020 until the 7th of April 2021. In the timeframe 2020/2021, there were 2186 deaths in the UHS, compared to an average of 1939 in the four preceding years, with a statistically significant difference between the two. This result goes in hand with the WHO predictions of an excess mortality caused by SARS-CoV-2 (40). There has been a noticeable steady downward trend of hospital deaths in the past years. When looking at the distinct years, it becomes apparent that while the number of deaths in the 2020/2021 period was higher than previous years, it was not notably higher than that in the year 2016/2017. Such fluctuations in annual mortality are commonly caused by variations of the seasonal influenza incidence (61).

The vast majority of fatal SARS-CoV-2 infections in Split occurred in the elderly population, with 87.17% of fatal COVID-19 cases being ≥65 years of age. Looking at non-COVID-19 hospital deaths, 78.93% were ≥65 years. Nguyen *et al.* also describe an increased in-hospital mortality with increasing age among COVID-19 patients (62). We further found a statistically significant increased mean age among the COVID-19 hospitalized fatalities compared to non-COVID-19 hospital death cases. Increasing age is one of the main risk factors for increased susceptibility to various infectious diseases. A recent systematic analysis has shown that increased susceptibility for severe disease course starts to rise long before old age. Individuals under 20 years of age are almost half as susceptible to COVID-19 as those over 20 (63). There have been indications that age-related susceptibility is more pronounced in COVID-19 than in other infectious diseases such as seasonal influenza (64). Moderbacher *et al.* discovered an uncoordinated adaptive immunity correlating with increased disease severity in elderly patients infected by SARS-CoV-2, characterized by an overall reduction of CD4+ and CD8+ T cell responses, loss of coordination of CD4+ and antibody responses as well as substantial cytokine responses. While an uncoordinated antigen-specific adaptive immune response was observed in patients ≥65 years, this response was even more severely altered in patients ≥75 of age. Such uncoordinated immunologic responses were frequently found to fail to control the infection (65). Thus, it can be speculated that these alterations in the course of SARS-CoV-2 infections are one of the reasons why older people more commonly fail to compete with this novel coronavirus. Another major factor that cannot be ignored is that the prevalence and severity of comorbidities increase with advanced age, while it has been shown that comorbid patients are at a marked increased risk for more severe disease progression and

worse outcomes of COVID-19 (52,66). Tiruneh *et al.* have also reported a significantly increased occurrence of COVID-19 complications in the elderly, some of them exhibiting a linear increased incidence with increased patient age (50). This increased risk of severe outcomes in the high age population should allow discussions about the introduction of medical as well as NPIs for specific age groups, as is the case in Sweden, where strict restrictions applied for those above age 70 and increased awareness of the population to protect such susceptible fellow citizens, was enforced (67). With emerging new variants current vaccine protection from infection and symptomatic illness recently dropped to 64%, but their efficacy in preventing serious illness and hospitalization remains at 93% (68). Therefore SARS-CoV-2 vaccination, especially regarding future variants, would likely be most effective in reducing mortality of COVID-19 when used primarily in high-risk patients.

We calculated a crude hospital mortality for patients with SARS-CoV-2 infection of 21.64%. Richardson *et al.* conducted a study among hospitalized patients with COVID-19 in New York and reports a mortality of 24.5% (48). A recent meta-analysis identified a mortality rate of admitted patients of 17.1% (95% CI 12.5; 22.7), which overlaps with our result (69).

For our preliminary estimation of the COVID-19 CFR, we calculated a crude CFR of 1.62% for the Split-Dalmatia-county. Crude overall CFRs of Israel, South Korea, Spain, and Italy accounted for 0.82, 2.26, 8.02, and 14.20 respectively. It is widely known that it should be avoided to compare crude CFRs between different countries and if comparisons are made, age-specified and age-adjusted CFRs should be used (70). A commonly discussed problem with CFR values is their subjectivity and room for error, most importantly due to imprecise nominators owing to nonunified diagnostic and tracing approaches and the general underestimation of infected cases. This is a known phenomenon, which is especially pronounced in diseases that can have asymptomatic or mild courses, mimicking common upper airway infections, leading to cases being misdiagnosed or not getting identified (3). This was already described with MERS, leading to an overestimation of the mortality, and has been confirmed for COVID-19 in multiple studies comparing seropositivity with reported cases (21,71). Another prerequisite for accurate determination of any mortality estimate is the question of how many of the patients really died from the disease. German pathologist Püschel points out that after the first initial months only a few deaths were causally attributable to COVID-19, with a mortality of less than 0.5% in Germany. Especially elderly individuals aged 70-90 had an advanced internal illness, resulting in fatal SARS-CoV-2 infections. On average, it was calculated that such fatal cases had lost 10 years of life expectancy (72). Similar

evaluations require further studies, as the average life expectancy in Croatia from the last demographic statistics in 2018 amounted to 78.2 years, while the median age of the COVID-19 fatalities was 79. Life expectancy by gender puts the males at 74.9 and females at 81.4 years while the median age of COVID-19 fatalities was 82 years for females and 77 years for males (73). Future studies could compare the mean age at death of endemic diseases and compare them to COVID-19 and the population's life expectancy.

Limitations to this study must be considered when interpreting the conclusions. Since the coroner's data regarding citizens deceased outside of the cities hospital system are not digitalized, we had to limit the collection of information to the UHS and the main city cemetery and morgue, which was the designated place for the burials of the whole city's COVID-19 (hospital and non-hospital) cases. Even though the UHS was the county's designated COVID-19 center, there could have been critical COVID-19 patients with fatal outcomes outside of Split, which would not have been included in our collection of data. When interpreting yearly crude numbers of deceased in the hospital, it has to be kept in mind that we did not gather numbers of the deceased outside the hospital for the same time periods. While there was an increase of total hospital deaths in the period 2020/2021, it requires further investigations into the deaths occurring outside the hospital to exclude absolute increases and decreases of mortality in the city of Split. Furthermore, excess death is usually described in weekly numbers, but due to limitations in data collection, we were only able to get crude numbers for yearly periods. One critical question often asked is whether patients actually died from COVID-19, or rather with it. In regard to the advanced age of fatal cases, a considerable critical general health condition due to high age, previous and pre-existing diseases cannot be excluded. Autopsies are crucial in this pandemic, enabling a more differentiated assessment of mortality and precise determination of the cause of death. There is great reluctance to perform autopsies on COVID-19 deceased patients due to safety aspects, but the risk of infection by infected deceased patients may be overestimated, while the scientific benefit gained from such post mortem studies is immeasurable (74). As the renounced German pathologist Prof. dr. Püschel put it, *mortui vivos docent*. To learn from the dead … for the living (72). To this date, the local coroners were not able to conduct a post-mortem examination on even a single SARS-CoV-2 patient due to imposed safety regulations for dissection of infected bodies. Hence, at this moment, a differentiation of cases that died with and those that really died of COVID-19 is not possible.

In conclusion, despite the above-mentioned limitations, our retrospective study was able to identify that the majority of fatal cases with COVID-19 occurred in individuals aged ≥65 years and there was a statistically significant higher mean age of COVID-19 fatal hospitalized cases compared to non-COVID-19 hospital fatalities. We did find an increased overall hospital mortality in the UHS in the time period 2020/2021, compared to previous years. Though, similar numbers were also present in selected previous years with no pandemic, possibly caused by a severe influenza season. A first crude CFR estimate of 1.62% for SARS-CoV-2 in the Split-Dalmatia-county and a hospital mortality of 21.64% was identified. The most widespread pandemic of the past century caused by SARS-CoV-2 will likely have an impact on our lives for years to come, in one way or another. While this study only investigated epidemiological data concerning fatal COVID-19 cases, we do hope it paves the way for more local studies investigating the risk of mortality by SARS-CoV-2.

6. CONCLUSION

The evidence provided by this study confirms both of our hypotheses from the beginning.

Our first hypothesis was that there was an increased in-hospital mortality in the first year after the initial COVID-19 fatality in Split, Croatia. The hypothesis was proven correct since the number of hospital deaths in the period from the 7th of April 2020 until the 7th of April 2021 was significantly higher than the mean number of hospital deaths from the previous four years.

Secondly, we confirmed the second hypothesis that over 80% of all fatal cases of SARS-CoV-2 infections occurred in patients ≥65 years old. Our analysis shows that 87.17% of hospital COVID-19 fatalities were ≥65 years old, compared to 78.93% of non-COVID-19 hospital deaths. These results reinforce the fact that advanced age is an important risk factor for fatal COVID-19 outcome and attempts to effectively lower mortality by SARS-CoV-2 should be focused on risk patients.

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8. SUMMARY

Objectives: The objective of this study was to investigate the mortality of fatal cases of SARS-CoV-2 in the first year from the initial COVID-19 casualty in Split, Croatia.

Materials and Methods: This was an observational cross-sectional study. Data was collected by reviewing the coroner logbooks of the UHS and the city of Split coroners, as well as the UHS database. The study captured the period from 7 April 2020 until 7 April 2021, a one-year timeframe from the initial COVID-19 case of death in Split. The sample for this study consisted of 2222 subjects, 36 COVID-19 positive patients which died outside of the hospital, 572 COVID-19 positive patients which died while hospitalized, and 1614 COVID-19 negative patients which died in the UHS. Factors collected include age, gender, date of death, and prior hospitalization.

Results: An increased number of hospital deaths was found for the period 2020/2021 compared to the mean of the previous four years. The majority of deceased patients with SARS-CoV-2 infection were aged ≥65 years. A crude COVID-19 CFR of 1,62% and a hospital mortality of 21,64% was estimated.

Conclusions: Fatal hospitalized cases of COVID-19 had a statistically significant higher mean age compared to non-COVID-19 hospital deaths. The elderly are at an increased risk of death during infection with SARS-CoV-2, and attempts to lower COVID-19 mortality should be focused on such risk patients. Further studies are required for a more accurate estimation of the increased risk of mortality.

9. CROATIAN SUMMARY

Naslov: Smrtnost od SARS-CoV-2 u Splitu, Hrvatska

Ciljevi: Cilj ovog rada je istraživanje mortaliteta smrtnih slučajeva SARS-CoV-2 u prvoj godini, od prvog slučaja COVID-19 u Splitu, Hrvatska.

Materijali i metode: Ovo je bilo observacijsko presječeno istraživanje. Podaci su prikupljeni pregledom mrtvozorničke knjige KBC Splita i mrtozornika grada Splita te baze podataka KBC Splita. Studija obuhvaća razdoblje od 7. travnja 2020 do 7. travnja 2021, jednogodišnji raspon od prvog smrtnog slučaja u Splitu. U studiju je uključeno 2222 pacijenata, 36 COVID-19 pozitivnih pacijenata koji su umrli izvan bolnice, 572 COVID-19 pozitivnih pacijenata koji su umrli u bolnici, i 1614 COVID-19 negativnih pacijenata koji su umrli u Kliničkom bolničkom centru Split u promatranom razdoblju. Prikupljeni podaci uključuju dob, spol i datum smrti.

Rezultati: Utvrđen je povećan broj bolničkih smrti u 2020/2021. godini usporedno sa prosjekom prijašnje četiri godine. Većina umrlih pacijenata sa SARS-CoV-2 infekcijom su imali 65 ili vise godina. Okvirni omjer smrtnih slučajeva (CFR) u Splitsko Dalmatinskoj županiji COVID-19 infekcije je 1m62%, a bolnička smrtnost hospitaliziranih COVID-19 pacijenata procijenjena je 21,64%.

Zaključci: Hospitalizirani smrtni slučajevi COVID-19 su imali statistički značajno veći prosjek godina usporedno sa COVID-19 negativnim bolničkim smrtnim slučajevima. Starije osobe imaju povećan rizik smrti tijekom infekcije SARS-CoV-2 i pokušaji da se smanji smrtnost COVID-19 bi se trebali fokusirati na tu rizičnu skupinu pacijenata. Potrebne su daljne studije za točnije procjene povećanog rizika smrtnosti.

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10. CURRICULUM VITAE

Personal Data

Name: Joshua Niemeyer

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Education

2004-2013 Wildermuth Gymnasium Tübingen, Germany

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Training

Internships at Helios hospital Perlach; Munich, Germany

Surgical ward	2010
Emergency department	2011
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Radiology; patient admission	01-02/2017
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