Retrospective analysis of disease severity and symptoms across the SARS-CoV-2 variants

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Master's thesis / Diplomski rad

2023

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Split, School of Medicine / Sveučilište u Splitu, Medicinski fakultet**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:171:515659

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RETROSPECTIVE ANALYSIS OF DISEASE SEVERITY AND SYMPTOMS ACROSS THE SARS-COV-2 VARIANTS

Diploma thesis

Academic year:
2022/2023

Mentor:
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Coburg, July 2023

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Acknowledgment

First and foremost, I would like to thank those who made it even possible that I can be a part of this study – my parents. Thank you for your continuous support since 1998, especially in the last 6 years.
Without both of you, I would never have the chance to pursue my dream.

I also want to express my gratitude to all those people, who were in the right place at the right time when encouragement was needed.

Finally, many thanks to Dr. Claus Steppert who made this thesis possible and to Prof. Dr. Johannes Brachmann, for always having an open ear for his students and his continuous dedication to our Medical School.

Last but not least, special thanks from the bottom of my heart to my grandmother. You always rooted for me till your last day.

List of Abbreviations

ACE2 – angiotensin converting enzyme 2
ARDS – acute respiratory distress syndrome
CBC – complete blood count
COPD – chronic obstructive pulmonary disease
COVID-19 – corona virus disease 2019
CRP – C-reactive protein
ELISA – enzyme-linked immunosorbent assay
ESR – erythrocyte sedimentation rate
FDA – food and drug administration
gRNA
HIV-1 – human immunodeficiency virus 1
IL-2
IL-4 – Interleukin 4
IL-5 – Interleukin 5
IL-6 – Interleukin 6
IgG – Immunoglobulin G
IgM – Immunoglobulin M
JAK – Janus kinase
JAK1 – Janus kinase 1
JAK2 – Janus kinase 2
JAK3 – Janus kinase 3
TYK2 – tyrosine kinase 2
JAKi – Janus kinase inhibitor
N protein – nucleocapsid protein
LFIA – lateral flow immunoassay
MERS-CoV – middle East respiratory syndrome coronavirus
PCT – Procalcitonin
R_0 – basic reproduction number
R_e – effective reproduction number
RAAS – renin-angiotensin-aldosterone system
RBC – red blood cells
RBD – receptor binding domain

RCT – randomized controlled trial

RdRp – RNA-dependent-RNA polymerase

RSV – respiratory syncytial virus

RT-PCR – real-time polymerase chain reaction

SARS-CoV – severe acute respiratory syndrome coronavirus

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2

S protein – spike protein

TMPRSS2 – transmembrane serine protease 2

VBM – variants being monitored

VOC – variant of concern

VOHC – variant of high concern

VOI – variant of interest

WBC – white blood cells

WHO – world health organization

1. INTRODUCTION

1.1. Evolution of the Covid-19 Epidemic

In December 2019 a novel coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in Wuhan, China, spreading globally to become a pandemic and being declared a public health emergency by World Health Organization (WHO) in March 2020 (1). It is the third documented spillover of an animal coronavirus to humans in only two decades, including two highly pathogenic coronaviruses with zoonotic origin, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) causing fatal respiratory illness (2,3).

The first known cases date back to 8 December 2019. Several health facilities in Wuhan reported clusters of patients with pneumonia of unknown cause, showing symptoms of viral pneumonia including fever, cough, and chest discomfort. Dyspnea and bilateral lung infiltration were reported in more severe cases. Epidemiologically, most cases were linked to Huanan Seafood Wholesale Market, which sells seafood and livestock - later, more cases were reported with no history of exposure to the Huanan Seafood Wholesale Market, including familial clusters of infection, as well as nosocomial infections in health-care facilities. In conclusion, this provided clear evidence for human-to-human transmission of the new SARS-CoV-2 virus (3).

On 23 January 2020 Wuhan City was under lockdown. Six days later, the novel coronavirus was reported to have spread across all 34 provinces in China and declared a public health emergency of international concern by WHO on January 30. The disease was designated as COVID-19 by the WHO, and the new coronavirus was given the name "SARS-CoV-2" by the International Committee on Taxonomy of Viruses on February 11 (3).

Despite the measurements taken by the Chinese Health Officials, global spread of COVID-19 continued, with large clusters of infections being reported from an increasing number of countries. The rapid worldwide spread was supported by international travel and the high transmission efficacy of SARS-CoV-2, resulting in a classification as pandemic by WHO on 11 March 2020 (3).

Up until 30 May 2023, 766.895.075 confirmed cases of Covid-19 have been registered by the WHO, including 6.935.889 reported deaths (4).

1.2. SARS-CoV-2 Genomics

SARS-CoV-2 is an extremely contagious respiratory virus causing adult atypical pneumonia COVID-19 with severe acute respiratory syndrome (SARS). SARS-CoV-2 is a single-stranded, positive-sense RNA-virus, belonging to the family of Betacoronavirus genus (5).

All human coronaviruses are believed to be a result of zoonotic transfer ("spillover") from animal reservoirs, which occurred either directly or through an intermediate animal host with bats being the reservoir of most coronaviruses. The genome of SARS-CoV-2 is 96.2% identical to a bat coronavirus RaTG13 from Yunnan province of Southern China, supporting the hypothesis of a possible bat origin. Additionally, those findings were further supported by another SARS-CoV-2-like bat coronavirus genome, RpYN06, exhibiting 94.5% sequence identity to the SARS-CoV-2 genome (5).

The SARS-CoV-2 genome is packed by viral nucleocapsid (N) proteins and enclosed by an envelope membrane with lipids and viral proteins (surface or spike), M (membrane) and E (envelope). The S protein specifically binds to a cellular receptor, angiotensin-converting-enzyme 2 (ACE2), thus enabling viral entry into susceptible cells and initiating the first step of virus infection. As a S protein activation protease serves the host cell transmembrane serine protease 2 (TMPRSS2), which due to its proteolytic cleavage of the S protein, initiates the fusion of viral and host membrane, as well as release of the viral gRNA into the cytoplasm. ACE2 and TMPRSS2 are expressed widely in a variety of cell types but with particular high expression in lungs, intestinal epithelia and endothelial cells, enabling SARS-CoV-2 to target multiple vital organs. Currently, the spike-protein is regarded as the only structure targetable by neutralizing antibodies, specifically the receptor binding domain (RBD) of S protein being a potential target for developing vaccines and drugs, as it has antigenic properties and could block the RBD-hACE2 interaction (5–7).

1.3. SARS-CoV-2 Variants and Variants of Concern

Since the first documented cases of COVID-19 in December 2019, multiple genetic lineages of SARS-CoV-2 occurred, which are routinely monitored through epidemiological investigations, virus genetic sequence-based surveillance, and laboratory studies.

Five mutated strains mainly occurred, termed alpha, beta, gamma, delta, and omicron. The first discovered spike protein (S protein) mutation D614G swept the world in July 2020 and was followed two months later by the alpha variant discovered in the United Kingdom. In December 2020 the beta variant was discovered in South Africa, in January 2021 gamma variant in Brazil, in March 2021 delta variant in United Kingdom and Omicron variant in November 2021 in Botswana (8).

The first sporadic cases in Germany appeared at the start of 2020, resulting in the first COVID-19 wave, which began in March 2020 and ended in September 2020, followed by the second COVID-19 wave, which lasted until the end of February 2021. The Alpha variant (B.1.1.7) was the cause of the third COVID-19 wave, which lasted from March 2021 until the beginning of August 2021. The fourth wave, which was brought on by the Delta variant (B.1.617.2), began to manifest in August 2021 and persisted through the end of December 2021. Subsequently, the fifth wave followed, which was caused by the Omikron variant (B.1.1.529), lasting from the end of December 2021 up until May 2022 (9).

Emerging variants are classified in order to monitor their potential impact on vaccines, therapeutics and diagnostics, as well as transmission and severity of the disease. Classification includes four types: variant of interest (VOI), variant of concern (VOC), variant of high consequence (VOHC) and variants being monitored (VBM). Variants designated as VOI include variants that show changes to their receptor binding domain (RBD), reduced neutralization by antibodies against previous infection or vaccination, reduced efficacy of treatments or tests, or with a predicted increase in transmissibility or disease severity. Variants designated as VOC show, additionally to the same characteristics as VOI, an increase in transmissibility, more severe disease course, a significant reduction in neutralization by antibodies generated during previous infection or vaccination, as well as reduced effectiveness of treatments or vaccines, or diagnostic detection failures (10).

In Germany several different variants of concern occurred – from the beginning of December 2020 Alpha B.1.1.7, Beta B.1.351 and Gamma P.1, in May 2021 Delta B.1.617.2 and from November 2021 on Omicron B.1.1529 (11).

1.4. SARS-CoV-2 Transmission

Transmission of SARS-CoV-2 from person to person is primarily through exposure to respiratory fluids and occurs in three ways: respiratory droplets and aerosol particles which are inhaled; direct splashes and sprays that deposit respiratory droplets and particles on exposed mucous membranes; contact of mucous membranes with hand that have previously been contaminated. The risk for infection depends on the amount of virus to which a person is exposed, therefore the risk for infection is decreased when there is an increased distance from the source and with increased time after exhalation. Infections from inhalation are less likely at distances greater than six feet but can still occur under certain circumstances (12).

In order to accurately determine the capability of transmission of SARS-CoV-2 it is important to define the reproductive number (13). The basic reproduction number (R_0) describes the number of secondary infections that result from a single index infection in a population that is otherwise susceptible. It has been widely used as measurement for the epidemic risk of SARS-CoV-2 and is an important tool for rating preventive measures and testing strategies (14). It also enables to estimate the proportion of a population that needs to be immune in order to prevent an epidemic (15). R_0 can be used to predict the level of immunization required to attain herd immunity. In order to stop the sustained spread of infection, the proportion of the population that needs to be immunized (P_i) has to be $>1-1/R_0$ (16). An R_0 of 1 or below indicates the end of an epidemic outbreak, whereas a number greater than 1 indicates a growing epidemic (17). The pooled R_0 for COVID-19 was estimated as 3.32 (95% confidence interval, 2.81 to 3.82) (18). In conclusion, this reinforces the notion that COVID-19 is a highly contagious disease, by showing that one SARS-CoV-2 infected person is likely to infect 3 persons (19).

The effective reproduction number (R_e), also known as R_t, is the number of people in a population who can be infected at any given moment by a single person. When we measure the transmissibility of the virus throughout the epidemic, we use R_e. It alters when the population becomes increasingly immunized, either through individual immunity after infection or following vaccination or when people die. It is influenced by number of infected people, the number of susceptible people they are in contact with and protective measurements such as social distancing (16).

1.5. SARS-CoV-2 Laboratory Diagnostics

As a detection method for large scale screening non-invasive sampling is preferred. Salivary glands, gingiva, oral mucosa, and the tongue show high expression of hACE2 receptors, therefore serving as hosts for SARS-CoV-2 and making saliva a good sampling choice. Additionally, Realtime-PCR (RT-PCR) using saliva samples may be used. IgM and IgG antibodies against SARS-CoV-2 S protein can be detected by lateral flow immunoassay (LFIA) within 15 minutes from blood samples, with a sensitivity of 88.66% and specificity of 90.63%. IgM antibodies are detected between 5 and 10 days, whereas IgG is detected between 14 to 21 days. Detection of IgM and IgG antibodies against nucleocapsid protein and S protein from confirmed COVID-19 patients showed that ELISA-based diagnostics has a significantly higher rate of positive results (7). Still, RT-PCR in sputum samples remains the gold standard for the diagnosis of COVID-19 (20).

Inflammatory responses play a major role in the development of COVID-19. Inflammatory parameters include Interleukin-6 (IL-6), C-reactive protein (CRP), procalcitonin (PCT), serum ferritin and erythrocyte sedimentation rate (ESR), which have been linked to a high risk for the development of severe COVID-19 (21).

A key immunomodulatory cytokine is IL-6, which is secreted by T cells and required for antibody production by B cells; it additionally affects the pathogenesis of diverse diseases (22,23). Increased levels of interleukins in COVID-19, especially IL-6, are seen in a “cytokine storm”, a profound systemic increase of inflammatory mediators and cytokines (24). High blood levels of IL-6 can be used as an important prognostic information on mortality and disease severity (25). Lower levels of IL-6 were detected in COVID-19 survivors compared to the IL-6 levels of non-survivors (21).

Serum C-reactive protein (CRP) level measurement is frequently used in clinical settings as a sensitive indicator of inflammation. It plays a role in combating bacteria, clearance of apoptotic cells and is a key part of the human acute-phase response (26). Evaluation of CRP in hospitalized COVID-19 patients may help to identify cases with an unfavorable prognosis, since elevated levels of CRP are significantly associated with COVID-19 severity (24,27).

Leukocytes, also called white blood cells (WBC), are part of the immune system and required for innate and humoral immune responses. Clinically, leukocytes are measured by the complete blood count (CBC), a test which also includes red blood cells (RBCs) and platelets (28). WBC counts were considerably higher in patients with severe and fatal cases of COVID-19 (29).

D-Dimer is a laboratory parameter used in the detection of thrombosis and inflammation (30,31). In the early stages of COVID-19 disease elevated D-dimer levels may be detected, with a 3 to 4-fold-increase being linked to a poor prognosis. Its levels may be influenced by certain underlying disorders such as diabetes, malignancy, stroke, and pregnancy. Therefore, the measurement of D-dimer and coagulation parameters in the early stage of COVID-19 can be important in management of the disease (31).

Vitamin D is a fat-soluble vitamin, which has an important role in inflammation and immune function. In particular, vitamin D regulates innate and adaptive immunity, cytokine release and inflammation and may lower the risk of infections. Supplementation of Vitamin D reduced the incidence of acute respiratory infections, including influenza infection, by 12%. Therefore, it may have a possible protective role in COVID-19 infections (32). Moreover, vitamin D deficiency increased hospitalization and mortality from COVID-19 (33).

1.6. Clinical presentation of COVID-19

COVID-19 presents itself in a various pattern and affects multiple organs. Manifestation depends on the number of expressed ACE-2-receptors in tissue (34). COVID-19 can cause complications through direct or indirect mechanisms, including viral toxicity, dysregulation of the renin-angiotensin-aldosterone system (RAAS), endothelial cell damage and thromboinflammation, cytokine storm and oxygen supply-demand mismatch (35).

Symptom onset is around 2-14 days after exposure to SARS-CoV-2 and may range from mild symptoms to severe illness requiring hospitalization (36). Asymptomatic infections may occur, in which patients are tested positive for the presence of SARS-CoV-2 by RT-PCR but are lacking typical clinical symptoms or imaging abnormalities. Asymptomatic patients are still able to transmit the virus, requiring early recognition in order to stop transmission (37).

Most common symptoms include fever, cough, myalgia, fatigue, and dyspnea. Common gastrointestinal symptoms include diarrhea; abdominal pain and vomiting were reported less commonly (38,39). Neurological symptoms include headache, changes in taste and smell, vertigo, and confusion. Additionally, neuropsychiatric manifestations including encephalitis, stroke, Guillain-Barré- and Miller-Fisher-syndrome are described (40). Cardiovascular manifestations include myocarditis, acute myocardial infarction, cardiac insufficiency, arrhythmias, and thromboembolic events (41). Hypercoagulability is associated with severe courses of COVID-19 leading to an increased risk for thromboembolic events, including deep vein thrombosis, pulmonary embolism and cerebrovascular events (42,43).

Patients with a severe clinical course may develop a potentially life-threatening hyper-inflammatory condition 8-15 days following the onset of symptoms, subsequently called cytokine-storm syndrome, resulting in multi-organ failure due to a dysregulated immune response leading to inappropriately elevated proinflammatory cytokines and chemokines (44,45).

The existence of a variety of symptoms with a duration beyond the acute phase of COVID-19, is referred to as post-COVID-19 syndrome (46). Also called Long COVID syndrome, it contributes significantly to morbidity in COVID-19 patients, estimated to affect approximately 43% of confirmed COVID-19 patients. It is characterized by a wide range of symptoms, including persistent fatigue, sensory deficits, neurocognitive deficits, as well as cardiovascular conditions such as myocarditis and arrhythmias that last for at least 2 months post-COVID and are unexplained by any other diagnosis. In the post-COVID phase common cardiovascular symptoms include chest pain, palpitations, shortness of breath with exertion, pre-syncope, fatigue, and pedal edema. The pathophysiology behind the cardiovascular manifestations has yet to be determined (47). Gastrointestinal symptoms, including loss of appetite, dyspepsia, irritable bowel syndrome, loss of taste and abdominal pain could commonly be seen as symptoms of long COVID (48). Neurological manifestations in post-COVID-syndrome include in particular fatigue, cognitive dysfunctions as brain fog, memory issues and attention disorder. Psychiatric manifestations are common, including sleep disturbances, anxiety, and depression, which increase significantly in prevalence over time (49).

1.7. Risk factors

As aforementioned, differ clinical presentations and disease severity in COVID-19 patients, ranging from asymptomatic to severe cases. In order to identify those high-risk patients, it is important to recognize the underlying predisposing factors contributing to a severe disease course (50). Serologic biomarkers can be used to determine severity in patients by the time they present clinically but early risk stratification, based on demographics and lifestyle factors, enables to improve patient outcomes and guide decision-making (50).

Important risk factor contributing to disease severity in COVID-19 include age >75 years old, male gender and severe obesity. Also, pre-existing medical conditions including hypertension, diabetes, cardiovascular diseases, malignancy, as well as a history of Chronic Obstructive Pulmonary Disease (COPD) and smoking, are contributing factors (50–52).

1.8. Treatment

Treatment guidelines of COVID-19 changed throughout the pandemic, due to rapidly growing evidence constantly changing practice recommendations (53). Currently available recommendations include non-drug treatment, such as various forms of ventilation, and additionally anti-inflammatory and immunomodulatory drug treatment options in later stages of COVID-19, including corticosteroids, Interleukin-6-receptor antagonists, or Janus kinase inhibitors. Additionally, early antiviral therapy options are now available, such as Remdesivir, Nirmatrelvir in combination with Ritonavir, and Molnupiravir. Due to their ineffectiveness or reduced efficacy against the currently dominant Omikron variant, SARS-CoV-2 neutralizing monoclonal antibodies, such as Casirivimab/Imdevimab, play only a minor role in the therapy of SARS-CoV-2 infections (54).

1.8.1. Anti-inflammatory and immunomodulatory drugs

1.8.1.1. Corticosteroids

Corticosteroids are drugs with anti-inflammatory and immunosuppressive properties, with dexamethasone being a major representative of long-acting synthetic corticosteroids. Other short-acting synthetic corticosteroids include methylprednisolone and hydrocortisone (55).

A high percentage of hospitalized patients with severe and critical COVID-19 develop respiratory failure requiring ventilatory support. An underlying imbalance between an insufficient host defense and inflammation seem to play a key role in the development of hypoxemic respiratory failure. An excess release of cytokines and inflammatory mediators contribute to the development of lung injury and acute respiratory distress syndrome (ARDS) (55). In comparison with standard care, the combination of interleukin-6 receptor antagonists with systemic corticosteroids reduces the risk of mechanical ventilation (odds ratio 0.79, 0.63 to 0.98; moderate certainty) (56). Therefore, current guidelines generally advise the administration of dexamethasone in hospitalized patients with severe symptoms, who are requiring oxygen (57).

1.8.1.2.Interleukin-6-receptor antagonists

Interleukin-6 (IL-6) plays a major role in severe to critically ill patients with COVID-19. Particularly its overproduction leads to an increase in disease severity and mortality. Therefore, a control in IL-6 overproduction and subsequent cytokine blockage plays an important role in disease control (58).

Tocilizumab, an IL-6-receptor-antagonist, can be administered in patients requiring high-flow supplemental oxygen or mechanical ventilation (58). Current guidelines recommend the administration of Tocilizumab in hospitalized patients requiring oxygen therapy, which show signs of hyperinflammation (C-reactive protein >75 mg/l), rapid clinical deterioration and are under ongoing dexamethasone therapy (57). Administration of IL-6 antagonists is associated with a decreased 28-day mortality in hospitalized COVID-19 patients (59).

1.8.1.3.Janus kinase inhibitors

As aforementioned, cytokines are key mediators of the immune response and virus clearance. Their corresponding transmembrane receptors are coupled to Janus kinases (JAKs), which are encoded by the human genome, and subsequently termed JAK1, JAK2, JAK3 and TYK2. When activated, JAKs are responsible for different processes, including proliferation, differentiation, and immune regulation. JAK inhibitors (JAKi) show, similar to corticosteroid therapy, immunosuppressive effects and therefore have the potential to interfere with pathological reactions in COVID-19. Several JAKi exist, such as baricitinib, ruxolitinib, tofacitinib and nezulcitinib. Currently, only baricitinib is recommended by the WHO as first-line agent in the treatment of patients with severe COVID-19 pneumonia, since it is the only JAKi proven to reduce mortality in individual randomized clinical trials (RCT) (60).

1.8.2. Antiviral therapy

Direct antiviral therapy acts by blocking viral replication and needs to be administered in the early phase of infection. In contrast to SARS-CoV-2-neutralizing monoclonal antibodies, which are directed against different epitopes at the receptor-binding-domains of the viral spike protein, the aforementioned direct viral agents are independent of the mutations in the spike protein (54).

Indication for antiviral therapy include the presence of risk factors for a severe course of infection, such as immunosuppression, age, or insufficient vaccination. Furthermore, onset of symptoms should not be more than 5 to 7 days ago (61).

1.8.2.1.Nirmatrelvir/Ritonavir (Paxlovid™)

Nirmatrelvir/ritonavir combines two medications with different mechanisms of action. Nirmatrelvir is a peptidomimetic inhibitor of Mpro, which is the main protease of SARS-CoV-2. It prevents the viral replication by blocking the procession of polyprotein precursors. The second component, Ritonavir, is a human immunodeficiency virus-1 (HIV-1) protease inhibitor with CYP3A-inhibiting effects. This enables it to decrease the CYP3A-mediated metabolism of nirmatrelvir. Coadministration of both agents is required in order to increase the plasma concentration of nirmatrelvir in order to achieve the targeted therapeutic range (62).

Nirmatrelvir/ritonavir shows effectiveness against SARS-CoV-2 and its major variants, including omicron (62).

1.8.2.2. Remdesivir (Veklury®)

Remdesivir belongs to the broad-spectrum antiviral agents and previously already demonstrated antiviral activity against filoviruses (Ebola viruses), coronaviruses (SARS-CoV, MERS-Co-V, SARS-CoV-2), paramyxoviruses (parainfluenza type III virus, mumps virus) and respiratory syncytial virus (RSV). The US Food and Drug Administration (FDA) approved Remdesivir as the first antiviral drug used in the clinical management of patients with severe COVID-19 (63).

Remdesivir is a phosphoramidite prodrug, which acts as a viral RNA-dependent RNA polymerase (RdRp) inhibitor and targets the viral genome replication process. In target cells it is metabolized into the pharmacologically active analog adenosine triphosphate, which competes with ATP at the RdRp complex, resulting in the termination of RNA synthesis and therefore blocking viral replication (63).

1.8.2.3.Molnupiravir (Lagevrio®)

Molnupiravir is a nucleoside-derived RdRp inhibitor, which acts primarily as a mutagenesis agent. It incorporates incorrect nucleo-bases into the viral genome, therefore inducing RNA mutations and leading to catastrophic errors. Newly synthesized virions continue the genomic errors, which interferes with their capacity of viral replication (64).

Effectiveness is proven against multiple viruses, including influenza A virus, Ebola virus, SARS-CoV and most recently SARS-CoV-2 (64).

Molnupiravir is approved by UK Medicines and Healthcare Products Regulatory Agency in the setting of severe cases of COVID-19 in adults, due to is potential to decrease morbidity and mortality in COVID-19 patients and ease disease severity (64).

1.8.3. Neutralizing monoclonal antibodies

In the European Union and Germany available SARS-CoV-2-neutralizing monoclonal antibodies show a reduced or absent in-vitro efficacy against the currently dominant Omikron-variant. Therefore, a monotherapy with monoclonal antibodies is currently not advised if other antiviral therapy options are available. If both Nirmatrelvir/Ritonavir and Remdesivir are not available as early antiviral therapy, Sotrovimab may be considered as a second line treatment option. Additionally, in cases of severe immunodeficiency, a combination of Nirmatrelvir/Ritonavir or Remdesivir and Sotrovimab may be considered (65).

1.8.3.1. Sotrovimab (Xevudy®)

Sotrovimab is a recombinant human monoclonal antibody directed against SARS-CoV-2 and binds to the RBD of the S protein. The first emergency use of Sotrovimab was authorized in the USA in May 2021 for the treatment of mild to moderate COVID-19 in patients who are at an increased risk for developing severe COVID-19. Administration is recommended to be within 5-7 days of symptom onset (57,66).

1.8.3.2.Tixagevimab/Cilgavimab (Evusheld®)

As aforementioned, Tixagevimab/Cilgsvimab is excluded from current treatment guidelines due to their markedly decreased in-vitro efficacy against Omikron-variants (61).

1.9. Vaccination and Prevention

The S protein is a good candidate for development of vaccines, antibodies, and drugs against SARS-CoV-2 due to its structural, functional, and antigenic characteristics. However, the Spike RBD of different Coronavirus strains shows a great antigenic capacity and undergoes frequent mutation, therefore always posing a risk of escape mutations. The majority of vaccines aim to prevent the uptake of viral particles via hACE2 receptors and to induce the formation of neutralizing antibodies. By using SARS-CoV-2 S protein in vaccine development it therefore serves two purposes: inhibition of receptor binding as well as viral genome uncoating. This could be achieved due to the distinct function of the Spike subunits, namely S1 and S2. S1 mediates hACE2 receptor binding, whereas S2 mediates the fusion and uncoating of the viral genome into the host cell (7).

Currently developed and released vaccines include Pfizer-BioNTech mRNA BNT162b2 (Comirnaty), Moderna mRNA 1273, Astrazeneca ChAdOx1-s (recombinant) and Janssen (Johnson & Johnson) (67).

1.9.1. Pfizer-BioNTech (Comirnaty) BNT 162b2 and Moderna (1273)

Both vaccines belong to the family of mRNA vaccines. They use genetically engineered RNA to generate a protein that safely prompts an immune response. The mRNA of the SARS-CoV-2 S protein was isolated, included in a lipid nanoparticle, and injected intramuscularly into the human body where it attaches to the host cells. This allows the mRNA to be inserted into the cytoplasm and leads to the synthesis of viral spike proteins by ribosomes. The synthesized proteins achieve a cellular membrane and start to attract antibodies against the viral spike proteins, as well as cells of the immune system, particularly T-helper cells, which produce cytokines including Interleukin-2 (IL-2), IL-4 and IL-5.

Pfizer and Moderna show an efficacy of 95-87.5% and 94.1% for preventing disease or severe disease (67,68).

1.9.2. Astrazeneca Oxford

The Astrazeneca/Oxford vaccine, called ChAdOx1-S (recombinant) is a viral vector vaccine, which uses a genetically engineered virus that encodes coronavirus proteins to safely generate an immune response. It uses a modified chimpanzee DNA adenovirus, that itself does not generate an immune response but only to the viral protein encoded in the host DNA. The DNA vector is able to encode a protein similar to the SARS-CoV-2 S protein, in order to generate an immune response against it.

For the Astrazeneca vaccine the efficacy was calculated as the capacity to prevent infection, not disease. Two studies were conducted in parallel in Brazil and in the United Kingdom, with the Brazilian study showing an efficacy of approximately 62% and the UK study 90%. Therefore, the mean efficacy value from both studies was 70% (67).

1.9.3. Janssen (Johnson & Johnson)

The vaccine of Janssen, called Ad.26.COV2.S or JNJ-78436725, acts through an engineered adenovirus, having a similar mechanism of action to that of the Astrazeneca vaccine.

Efficacy was measured by how well the vaccine prevented illness and showed a 65% efficacy respectively in preventing moderate and severe disease (67).

Since 6 May 2023 the vaccine is not available anymore in the United States (69).

1.9.4. Novavax

Nuvaxovid™, also termed NVX-CoV2373, is an adjuvanted recombinant SARS-CoV-2 spike trimer protein vaccine, which was granted conditional marketing on December 20, 2021, by the European Commission of the European Union (EU) (70,71). Currently, it is the first protein-based vaccine authorized as SARS-CoV-2 vaccine. It elicits both B-lymphocyte and T-lymphocyte immune responses to the S protein of SARS-CoV-2. The vaccine could protect against all SARS-CoV-2 viral variants due to the full-length S protein having common epitopes (71).

2. OBJECTIVES

2.1. Aims of the study

The aim of our study was to investigate if there is a difference in the presence of symptoms in different phases of COVID-19. Additionally, we explored potential differences in SARS-CoV-2 variants regarding their disease severity.

2.2. Hypothesis

There is a difference in disease severity regarding duration and type of hospitalization, and disease outcome between different SARS-CoV-2 variants. Additionally, the presence of symptoms and affected population vary between different SARS-CoV-2 variants.

3. MATERIALS AND METHODS

3.1. Ethical Approval

The plan of Research was approved by the Institutional Review Board of the Medical School REGIOMED Coburg on November 11, 2022.

3.2. Study design

The study was designed as a retrospective cohort study, conducted at the REGIOMED Hospital in Coburg, Bavaria, Germany.

Data, which was already collected from March 2020 until March 2022, was analyzed. The acquired data was anonymized and no conclusions about personal patient information can be drawn.

Based on data delivered from the Robert Koch Institute, patients were divided into respective study groups depending on the prevalent SARS-CoV-2 variant at the time of their positive PCR-result.

Table 1. Classification of phases during the COVID-19 pandemic in Germany

Phase	Variant of Concern (VOC)	Beginning (CW[a])	End (CW)
1	Wild type	10/2020	08/2021
2	Alpha	09/2021	30/2021
3	Delta	31/2021	51/2021
4	Omikron	52/2021	*

Data from the Robert Koch Institute
* End of Phase 4 not yet definable
[a] Calendar week
Steffen G, Behnke AS, Dudareva S. "Aktualisierte Phaseneinteilung der COVID-19 Pandemie". Epidemiologisches Bulletin. 2022;38.7-25.

3.3. Data collection

This retrospective study used the already collected and anonymized data of the REGIOMED hospital in Coburg. Additional information was acquired by the inbuilt search and filter option of the operating system *Orbis*. Collected variables included gender, age, date of admission and discharge, duration of hospital stay, reason for discharge, date of positive PCR-result, status of immunization and presence of symptoms at the time of admission. In the case of more than 7 days between date of admission and date of positive PCR-result, symptoms at the time of the positive PCR result were collected. Definition of symptoms included the presence of fever, cough, myalgia, dyspnea and/or fatigue, as well as the presence of anosmia and/or ageusia. Immunization status was defined by the number of immunization events, including vaccination, and passed SARS-CoV-2 infections and summarized by numbers 0-3. Cases without any immunization events were deciphered as number 0, number 1 was assigned in case of one event of immunization, number 2 with two events of immunization, whereas number 3 indicated individuals with three or more events of immunization. Discharged patients were categorized in different categories, including discharged home, transferred to another hospital, discharge into nursing facility or ventilator rehabilitation facility and in-hospital deaths. Additional collected variables included height, weight, BMI, admission to the intensive care unit, duration of stay on the intensive care unit and type of ventilator support.

Patients that had a positive SARS-CoV-2 PCR-test result and were hospitalized at the REGIOMED hospital in Coburg from 03/2020 until 03/2022 were included. Patients younger than 18 years of age, without available or negative PCR-test data or with a positive PCR-test at the day of discharge were excluded. The initial total number of cases included 1520 cases; 89 cases were dropped due to individuals being younger than 18 years of age.

3.4. Statistical Analysis

The statistical analyses were performed using Stata 16 (Stata Corp, College Station TX, USA). For presentation of data descriptive statistics including mean, median, standard deviation, minimum and maximum were used. Non-normally distributed data was compared with the Mann-Whitney U test. As an extension of the Mann-Whitney U test the Kruskal-Wallis test is used. Non-continuous data was presented in frequency tables and compared with a Chi-squared test. Statistical significance was set with a P value of <0.05.

4. RESULTS

4.1. Demographic characteristics of the study population

4.1.1. Gender distribution

In this study 1430 cases were included after considering the data collection listed above in Methods. Depending on the date of their positive PCR-result patients are categorized and assigned to the respective COVID-19 phases. Table 2 shows the gender distribution during the different phases, with phase 1 having the highest frequency with 600 cases in total, followed by Phase 4 with 335 in total. In total 731 males (51.1%) and 699 females (48.9%) were tested positive for SARS-CoV-2. Based on the provided data we could not suggest a significant association between gender and phase (P=0.145, χ^2=5.39, df=3).

Table 2. Gender distribution in COVID-19 phases

Gender	Phase 1	Phase 2	Phase 3	Phase 4	Total
Male	290	111	162	168	731 (51.1%)
Female	310	86	136	167	699 (48.9%)
Total	600	197	298	335	1430 (100%)

4.1.2. Age distribution

Table 3 provides data about the age distribution in the four phases of COVID-19. Considering all phases collectively, there are 1430 observations, with a mean age of 70.1. The age ranges from 18 to 101, with the median age being 74. To assess if there are significant differences in the median age across the phases, the Kruskal-Wallis test was conducted (χ^2=93.32, df=3, P=<0.001), indicating that statistically significant differences in the median age among the phases are present.

Table 3. Age distribution in COVID-19 phases.

Phase	N	Mean	SD	Min	Max	Median	Rank Sum*
1	600	74.86	15.66	18	101	79	496576.50
2	197	62.58	18.3	18	99	63	104963.50
3	298	69.81	15.87	22	97	73	204765.50
4	335	66.2	20.5	18	100	70	216859.50
Total	1430	70.09	17.9	18	101	74	-

*Kruskal-Wallis equality-of-populations rank test

4.1.3. Comparison of ages between genders

In males, the average age is 69.94 ± 15.95 years. The minimum observed age is 18, while the maximum is 101, and the median age is 73. For females, the mean age is 70.24 ± 19.73 years. The minimum and maximum ages are 18 and 100, respectively, and the median age is 76 (Table 4). The associated *P* of 0.011 indicates that there is a significant difference in age between the two gender categories, with males having a slightly higher average age compared to females.

Table 4. Age distribution depending on gender.

Gender	N	Mean	SD	Min	Max	Median	*P*
Male	731	69.94	15.95	18	101	73	
Female	699	70.24	19.73	18	100	76	0.011
Total	1430	70.09	17.89	18	101	74	

4.2. Presence of symptoms at admission

Characteristic symptoms of COVID-19 were predefined, as described in the data collection section, and noted at the time of admission. Symptoms were documented in a total of 134 cases, with 58 total cases in phase 1, 30 cases in phase 2, 23 cases in phase 3 and 23 cases in phase 4.

In Phase 1, 16 cases remained asymptomatic (27.59%), whereas 42 symptomatic cases are noted (72.41%). In Phase 2, 4 asymptomatic cases (13.33%) and 26 symptomatic cases (86.67%) are described. Phase 3 includes 15 symptomatic (65.22%) and 8 asymptomatic cases (34.78%). In Phase 4, 9 symptomatic (39.13%) and 14 asymptomatic cases (60.87%) are noted (Figure 1). Indicated by the percentages, Phase 2 shows the highest percentage of symptomatic cases, followed by Phase 1 and 3. In contrast, Phase 4 shows the highest percentage of asymptomatic cases. There is a significant correlation between the presence of symptoms at time of admission and corresponding phases (χ^2=14.34, *P*=0.002).

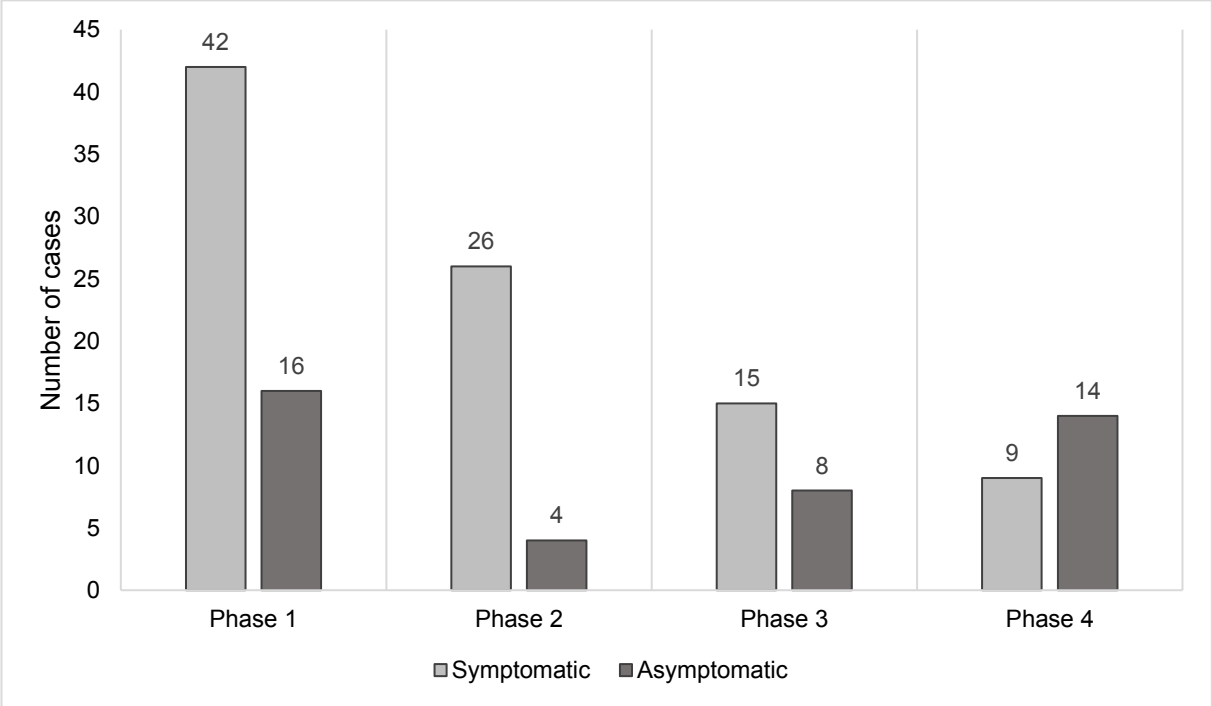


Figure 1. Presence of symptoms at the time of admission across different phases of COVID-19.

4.2.1. Gender and age distribution

The average age distribution for males in correlation with the presence of symptoms at the time of admission is presented in Table 5. For males, which represent without symptoms at admission, the mean age is 70.85 ± 16.24, ranging between age 20-101 and a median of 73 years. For symptomatic males, the mean age is 69.82 ± 15.48 years with the age ranging from 23-100 years and a median of 72.5. In comparison, the average age for males with no symptoms at admission is slightly higher than those without symptoms at admission. However, the difference is not substantial as mean ages are quite close (Table 5). Based on the Wilcoxon rank-sum test, there is no statistically significant difference in ages between individuals with no symptoms at admission and those with symptoms at admission (z=1.057, *P*=0.29). Therefore, we cannot conclude that the presence of symptoms at admission is associated with a significant difference in age.

Table 5. Average age distribution for males in correlation with the presence of symptoms at the time of hospital admission.

Symptoms at time of admission	Mean	SD	Min	Max	Median	*P*
Asymptomatic	70.85	16.24	20	101	73	0.29
Symptomatic	69.82	15.48	23	100	72.5	

The average age distribution for females in correlation with the presence of symptoms at the time of admission is presented by Table 6. For asymptomatic females, the mean age is 75.07 ± 16.57 years. Minimum age is 18 years and maximum age 100 years. The median age is 81 years. For symptomatic females the mean age is 71.04 ± 18.13 years. 22 years and 100 years are the minimum and maximum age, respectively. Median age is 75. In comparison, asymptomatic females tend to have a slightly higher mean age and median compared to symptomatic females. However, symptomatic females have a higher standard deviation, indicating a wider spread of ages within that category. The Wilcoxon rank-sum test indicates, that there is a statistically significant difference in ages between asymptomatic females and symptomatic females (z-value=3.07, *P*=0.002). Therefore, we can conclude that the presence

of symptoms at admission is associated with a significant difference in this specific gender group.

Table 6. Average age distribution for females in correlation with the presence of symptoms at the time of hospital admission.

Symptoms at time of admission	Mean	SD	Min	Max	Median	*p*
Asymptomatic	75.07	16.57	18	100	81	0.002
Symptomatic	71.04	18.13	22	100	75	

4.3. Disease outcome across different COVID-19 phases

Out of the total 1430 cases, 867 were discharged home (60.63%). 67 cases were discharged into a rehabilitation facility (4.69%), 147 were transferred into another hospital (10.28%), 76 discharged into a nursing home (5.31%) and 3 into a ventilator rehabilitation facility (0.21%) and in total 270 cases died (18.88%).

In Phase 1 600 cases were treated at the REGIOMED hospital in Coburg, out of which 288 cases were regularly discharged (48%). 41 cases were discharged into a Rehabilitation facility (6.83%), 98 cases transferred to another hospital (16.33%) and 34 cases released into a nursing home (5.67%). 1 case was discharged into a ventilator rehabilitation facility (0.17%). Additionally, with 138 of cases and 23% Phase 1 shows to have the highest percentage of death.

In Phase 2 a total of 197 cases were released after treatment, with 143 cases discharged home (72.59%). Compared to the first phase, lower percentages of cases had to be released into other facilities (3.05%, 8.12%, 0.51% and 0%, respectively). Death occurred in 31 cases (15.74%).

A total of 298 cases were treated at the REGIOMED hospital Coburg in Phase 3. 182 cases were discharged home (61.07%), whereas 11 cases were discharged into a rehabilitation facility (3.69%) and 23 cases transferred to another hospital (7.72%). Compared to Phase 2 higher percentages of cases hat to be released into nursing homes and ventilator rehabilitation facilities (5.37% and 0.67%, respectively). 64 cases died (21.38%), placing Phase 3 as the second-highest wave regarding number of deaths.

335 cases in total were counted in Phase 4, out of which 254 cases were discharged home (75.82%). Phase 4 shows the lowest percentages regarding the release into rehabilitation facilities, transfer to other hospitals and ventilator rehabilitation facilities (2.69%, 2.99% and 0%, respectively), with the exception of the number of discharges into nursing homes. They account for 25 cases (7.46%). Regarding the number of deaths, Phase 4 shows the lowest percentage of all four phases with 11.04% (Table 7).

The Pearson Chi-square test was conducted, in order to assess the relationship between the outcome and corresponding COVID-19 phase, indicating a significant association (χ^2=119.43, df=15, *P*=<0.001).

Table 7. Number of discharges in different phases of COVID-19.

Outcome	Phase 1	Phase 2	Phase 3	Phase 4	Total	*P*
Discharged home	288 (48%)	143 (72.59%)	182 (61.07%)	254 (75.82%)	867 (60.63%)	
Rehabilitation Facility	41 (6.83%)	6 (3.05%)	11 (3.69%)	9 (2.69%)	67 (4.69%)	
Transferred	98 (16.33%)	16 (8.12%)	23 (7.72%)	10 (2.99%)	147 (10.28%)	
Nursing facility	34 (5.67%)	1 (0.51%)	16 (5.37%)	25 (7.46%)	76 (5.31%)	<0.001
Ventilator rehabilitation facility	1 (0.17%)	0 (0%)	2 (0.67%)	0 (0%)	3 (0.21%)	
In-hospital death	138 (23%)	31 (15.74%)	64 (21.48%)	37 (11.04%)	270 (18.88%)	
Total	600 (100%)	197 (100%)	298 (100%)	335 (100%)	1430 (100%)	

4.3.1. Form and outcome of inpatient care

Out of 1430 cases hospitalized at the REGIOMED hospital in Coburg during the time frame of March 2020 to March 2022, a total of 1271 cases required either treatment in an intensive care unit (ICU) or standard care unit (SCU). 159 cases were excluded due to missing data.

In Phase 1 104 cases were treated in the ICU (20,12%), whereas 413 cases (79,88%) required standard care. Phase 2 showed the highest percentage of cases requiring treatment in ICU, compared to other COVID-19 phases, with 50 cases respectively (26.32%). 140 cases required treatment in SCU (73.68%). 39 cases were hospitalized in the ICU in Phase 3 (14.23%) and 235 required SCU (85.77%). Phase 4 has the lowest percentage of cases requiring treatment in ICU with 6.55% (19 cases) and conversely the highest SCU percentage with 93.45% (271 cases) (Figure 2).

In order to determine a relationship between the two variables the Pearson Chi-square test was used, which suggested a significant association between phases and ICU status (χ^2=39.67, df=3, *P*=<0.001).

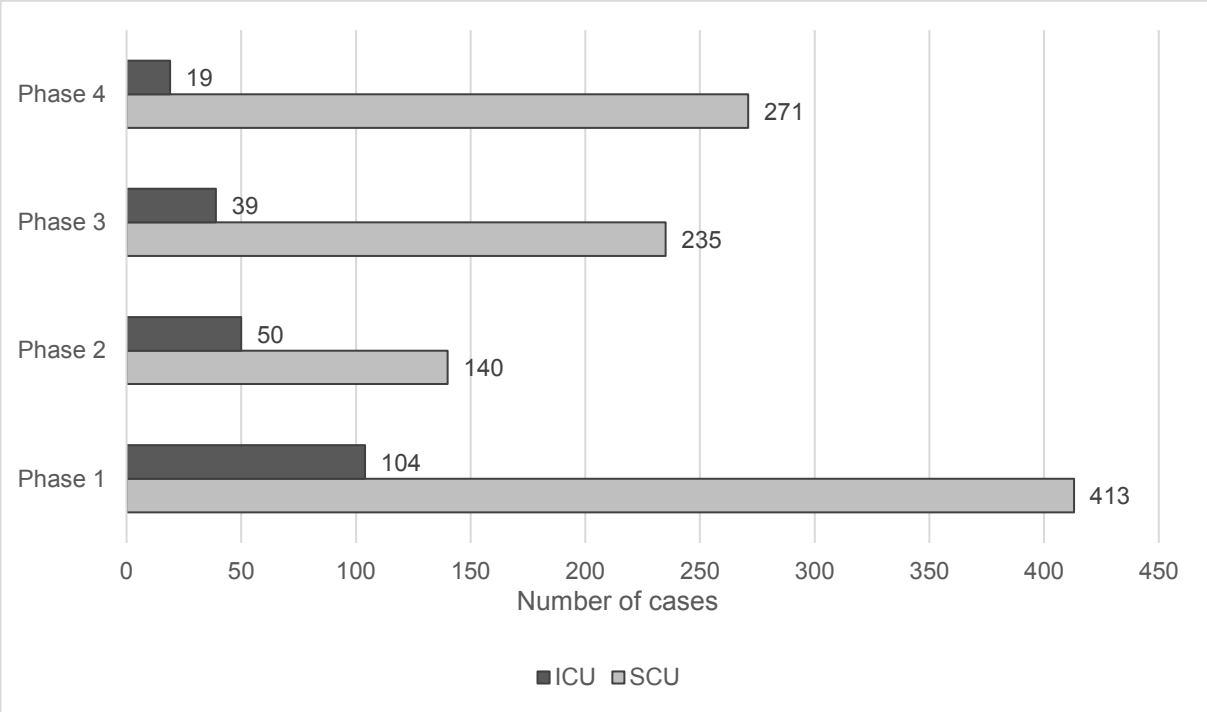


Figure 2. Number of admitted cases in the intensive care unit (ICU) *vs*. standard care unit (SCU) in the respective COVID-19 phases.

As aforementioned, a total of 1271 cases hospitalized at the REGIOMED hospital in Coburg required either treatment in an ICU or SCU. As displayed in Table 8, comparing the outcomes of cases hospitalized on the SCU vs. ICU, a higher home-discharge rate for SCU than ICU cases can be observed (66.38% vs. 24.06% respectively). Additionally, SCU cases showed lower discharge rates into specialized care facilities, including rehabilitation facilities or ventilation rehabilitation facilities (4.34% and 0%, respectively). Out of the total of 212 cases hospitalized on the ICU 109 died (51.42%), whereas out of the total 1059 SCU cases only 137 died (12.94%) (Table 8).

As statistical analysis the Pearson Chi-squared test was performed, showing a significant relationship between outcome and SCU/ICU status (χ^2=213.25, df=5, *P*=<0.001).

Table 8. Outcome of cases hospitalized on the SCU vs. ICU.

Outcome	**SCU[a]**	**ICU[b]**	**Total**	***P***
Discharged home	703 (66.38%)	51 (24.06%)	754 (59.32%)	
Rehabilitation Facility	46 (4.34%)	16 (7.55%)	62 (4.88%)	
Transferred	108 (10.20%)	28 (13.21%)	136 (10.70%)	
Nursing facility	65 (6.14%)	5 (2.36%)	70 (5.51%)	<0.001
Ventilation rehabilitation	0 (0%)	3 (1.42%)	3 (0.24%)	
In-hospital death	137 (12.94%)	109 (51.42%)	246 (19.35%)	
Total	1059 (100%)	212 (100%)	1.271 (100%)	

[a] Standard Care Unit
[b] Intensive Care Unit

4.3.2. Outcome in correlation with immunization status

The disease outcome in immunized and non-immunized cases is accounted for in Table 9 and 10. Immunization status is defined by the number of immunization events, including vaccination, and passed SARS-CoV-2 infections.

In total 625 cases were discharged home, out of which 351 individuals were non-immunized, 34 had a single event of immunization, 117 two events and 123 had three or more events of immunization. The column percentages indicate that 50.80% of the non-immunized individuals were discharged, compared to 66.67% of the single-immunized, 69.23% of the double-immunized and 78.85% of those with three or more immunization events (Table 9).

In the second category of cases discharged to rehabilitation facilities 56 cases were registered. 41 cases were non-immunized, 1 was single-immunized, 7 were double-immunized and 7 had three or more events of immunization. The column percentages show that 5.93% of the non-immunized individuals were discharged into a rehabilitation facility, compared to 1.96% of cases with one event of immunization, 4.14% of cases with two events of immunization and 4.49% of cases with three or more events of immunization (Table 9).

In total 116 cases were transferred to another hospital, including 99 cases who are not immunized. 2 cases were single-immunized, 9 were double-immunized and 6 had three or more events of immunization. The column percentages indicate that 14.33% of the non-immunized individuals required transfer, compared to 3.92% of single-immunized cases, 5.33% of the double-immunized and 3.85% of those who had three or more events of immunization.

In the category of cases discharged to a nursing facility, a total of 64 cases were reported, out of which 37 individuals were non-immunized, 6 were single-immunized, 11 were double-immunized and 10 had three or more events of immunization. The column percentages show that 5.35% of the non-immunized individuals required care, compared to 11.76% of the single-immunized, 6.51% of the double-vaccinated and 6.41% of the triple-immunized.

Only 3 cases were registered being discharged to a ventilator rehabilitation facility and all of them were from the non-immunized group.

A total of 203 in-hospital deaths were reported. Among them, 160 individuals were non-immunized , 8 were single-immunized, 25 were double-immunized and 10 had three or more immunization events. The column percentages indicate that 23.15% of the non-immunized individuals died, compared to 15.69% of the single-immunized, 14.79% of the double-immunized and 6.41% of those who had three or more events of immunization.

In summary, when considering the entire dataset with a total of 1.067 cases, a total of 691 individuals had zero events of immunization, 51 had one event of immunization, 169 had two events of immunization and 156 had three or more events of immunization.

In order to assess the statistical significance of the relationship between immunization status and outcome, a Pearson chi-square test was conducted, which indicated a highly significant association (χ^2=72.07, *P*=<0.001).

Overall, based on the provided data, there is an impact of the immunization status on the outcome of SARS-CoV-2 infections. Immunized individuals tend to have better outcomes, including higher discharge rates and lower mortality rates, compared to non-immunized individuals or those with fewer events of immunization.

Table 9. Disease outcome in immunized and non-immunized cases.

Outcome	**0**	**1**	**2**	**3**	**Total**	***P***
Discharged home	351 (50.80%)	34 (66.67%)	117 (69.23%)	123 (78.85%)	625 (58.58%)	
Rehabilitation facility	41 (5.93%)	1 (1.96%)	7 (4.14%)	7 (4.49%)	56 (5.25%)	
Transfer to another hospital	99 (14.33%)	2 (3.92%)	9 (5.33%)	6 (3.85%)	116 (10.87%)	
Nursing facility	37 (5.35%)	6 (11.76%)	11 (6.51%)	10 (6.41%)	64 (6%)	<0.001
Ventilator rehabilitation facility	3 (0.43%)	0 (0%)	0 (0%)	0 (0%)	3 (0.28%)	
In-hospital death	160 (23.15%)	8 (15.69%)	25 (14.79%)	10 (6.41%)	203 (19.03%)	
Total	691 (100%)	51 (100%)	169 (100%)	156 (100%)	1067 (100%)	

0=Non-immunized
1=One event of immunization
2=Two events of immunization
3=Three or more events of immunization

Table 10 provides us with a detailed analysis of the number of in-hospital deaths in immunized and non-immunized cases, subdivided according to the COVID-19 phase in which they occurred.

In Phase 1, a total of 138 in-hospital deaths occurred and 462 cases survived. Out of the 138 in-hospital deaths, 126 occurred in non-immunized individuals and 12 in individuals with three or more events of immunization. The column percentages show that 24.75% of non-immunized individuals died, whereas 75.25% survived, compared to individuals with three or more events of immunization, win which only 13.64% died and 86.36% survived (Table 10). As statistical test the Pearson chi-square test is used, indicating a significant association between immunization status and survival status (χ^2=6.14, df=2, *P*=0.046).

In Phase 2, a total of 31 deaths occurred, with 166 survivors. Out of the 31 in-hospital deaths, 5 deaths occurred in non-immunized individuals, 2 in cases with one event of immunization and in 24 cases of individuals with three or more immunization events. Looking at the column percentages, 84.38% non-immunized individuals survived in Phase 2, whereas 15.62% of them died. Regarding individuals with one event of immunization, 100% survived. In individuals with three or more events of immunization, 84.21% survived and 15.79% died (Table 10). The Pearson chi-square test was conducted, suggesting no statistical significant association (χ^2=0.42, df=3, *P*=0.935).

Phase 3 shows a total of 64 deaths, with 234 survivors. Out of the 64 deaths, 12 occurred in non-immunized individuals, 5 in individuals with one event of immunization and 23 in individuals with two immunization events. 19 cases were observed in individuals with three or more events of immunization. Checking column percentages, 81.52% of non-immunized individuals survived in Phase 3, whereas 18.48% of them died. In individuals with single immunization, 73.68% survived and 26.32% died. Double-immunized individuals show a survival rate of 80.83% and 19.17% died. In individuals with three or more events of immunization, 71.64% survived, whereas 28.36% died (Table 10). The Pearson Chi-square test indicates no statistically significant association (χ^2=3.02, df=3, *P*=0.389).

In Phase 4, a total of 37 deaths occurred with 298 survivors. Out of the 37 deaths, 12 occurred in non-immunized individuals, 1 in a single-immunized individual, 2 in double-immunized individuals and 22 in individuals with three or more events of immunization. The column percentages indicate, that in Phase 4 79.31% of non-immunized cases survived, whereas 20.69% died. Out of the single-immunized cases, 94.44% survive, whereas 5.56% died. 95.74% of individuals with two or more events of immunization survived Phase 4 and 4.26% died. Of those individuals with three or more events of immunization, 89.62% survived and 10.38% died (Table 10). The Pearson Chi-square test indicates a significant association (χ^2=8.34, df=3, P=0.039).

Table 10. Number of deaths in immunized and non-immunized cases in different phases of COVID-19.

Phase	Survival status	0	1	2	3	Total	*P*
Phase 1	Survived	383 (75.25%)	3 (100%)	-	76 (86.36%)	462 (77%)	0.046
	In-hospital death	126 (24.75%)	0 (0%)	-	12 (13.64%)	138 (23%)	
	Total	509 (100%)	3 (100%)	-	88 (100%)	600 (100%)	
Phase 2	Survived	27 (84.38%)	9 (81.82%)	2 (100%)	128 (84.21%)	166 (84.26%)	0.935
	In-hospital death	5 (15.62%)	2 (18.18%)	0 (0%)	24 (15.79%)	31 (15.74%)	
	Total	32 (100%)	11 (100%)	2 (100%)	152 (100%)	197 (100%)	
Phase 3	Survived	75 (81.52%)	14 (73.68%)	97 (80.83%)	48 (71.64%)	234 (78.52%)	0.389
	In-hospital death	17 (18.48%)	5 (26.32%)	23 (19.17%)	19 (28.36%)	64 (21.48%)	
	Total	92 (100%)	19 (100%)	120 (100%)	67 (100%)	298 (100%)	
Phase 4	Survived	46 (79.31%)	17 (94.44%)	45 (95.74%)	190 (89.62%)	298 (88.96%)	0.039
	In-hospital death	12 (20.69%)	1 (5.56%)	2 (4.26%)	22 (10.38%)	37 (11.04%)	
	Total	58 (100%)	18 (100%)	47 (100%)	212 (100%)	335 (100%)	

0=Non-immunized
1=One event of immunization
2=Two events of immunization
3=Three or more events of immunization

4.4. Oxygen requirements and type of ventilation

Table 11 displays the number of cases requiring oxygen during their hospital stay. In total 1217 cases were observed, with 500 cases occurring in Phase 1, 187 cases in Phase 2, 262 cases in Phase 3 and 268 cases in Phase 4.

In Phase 1, 222 cases did not require oxygen (44.40%). If oxygen was required, the most common type of ventilation required was oxygen via nasal cannula, which occurred in 186 cases (37.20%). 42 cases required non-invasive ventilation or High Flow Nasal Cannula (HNFC) (8.40%) and 50 out of a total of 500 cases required mechanical ventilation (10%).

In Phase 2 the majority of patients required oxygen via nasal cannula (72 cases, 38.50%), followed by 65 cases not requiring oxygen therapy (34.76%). 18 cases underwent non-invasive ventilation or HNFC (9.63%) and 32 cases mechanical ventilation (17.11%). In comparison, Phase 2 therefore showed the highest percentage of cases requiring oxygen via nasal cannula, mechanical and non-invasive ventilation.

112 cases did not require oxygen in Phase 3 (42.75%), followed by 96 cases receiving oxygen via nasal cannula (36.64%). In 30 cases non-invasive ventilation or HFNC was needed (11.45%) and 24 of cases had to be intubated (9.16%).

Phase 4 accounted for the highest percentage of cases which did not require oxygen with 189 cases and 70.52%, respectively. If they underwent oxygen therapy, it occurred most commonly via nasal cannula (56 cases, 20.90%). Compared to all other phases, Phase 4 shows the lowest percentage of cases requiring non-invasive ventilation (12 cases, 4.85%) and mechanical ventilation (10 cases, 3.73%).

To determine whether there is a statistically significant association between oxygen requirements and phases, the Pearson chi-squared test was performed. The result of $\chi^2=82.63$ with 9 degrees of freedom and a $P<0.001$ suggest a statistically significant relationship.

Table 11. Oxygen requirements and type of ventilation required in association with different phases of COVID-19.

Phase	No oxygen requirements	Nasal cannula	Non-invasive ventilation	Mechanical ventilation	Total	*P*
1	222 (44.40%)	186 (37.20%)	42 (8.40%)	50 (10%)	500 (100%)	
2	65 (34.76%)	72 (38.50%)	18 (9.63%)	32 (17.11%)	187 (100%)	
3	112 (42.75%)	96 (36.64%)	30 (11.45%)	24 (9.16%)	262 (100%)	<0.001
4	189 (70.52%)	56 (20.90%)	13 (4.85%)	10 (3.73%)	268 (100%)	
Total	588 (48.32%)	410 (33.69%)	103 (8.46%)	116 (9.53%)	1217 (100%)	

4.5. General duration of hospitalization

The duration of hospitalization was categorized and divided according to the respective phases, as seen in Table 12. A total of 1430 cases was analyzed, with a total of 600 cases in Phase 1, 197 in Phase 2, 298 in Phase 3 and 335 in Phase 4.

Looking at the summary statistics, we can see a variability in the mean duration across phases. Phase 1 has the highest mean hospitalization duration of 13.67 days, followed by Phase 3 with a mean of 12.82 days. Phase 2 has a slightly lower mean of 10.98 days and Phase 4 the lowest mean of 9.81 days. The variability within each phase is indicated by the standard deviation (SD), with Phase 1 having the highest variability (12.99) and Phase 4 having the lowest (9.74).

Differences in phases are also seen regarding the range of values (min and max). The minimum duration is 0 days collectively in all phases, indicating that there are cases released at the same day of admission. The maximum duration varies from 80 days in Phase 1 to 83 days in Phase 3. With 54 days Phase 4 has the lowest maximum duration.

A measurement of the central tendency of duration is provided by the median. A median duration of 9 days is seen in Phase 1 and 3, while Phase 2 has a slightly lower median of 8 days. With a duration of 6 days, Phase 4 has the lowest median.

The Kruskal-Wallis test was conducted to examine the equality of populations across the phases, which compares the rank sums of the observations in each phase. The results indicate strong evidence, that the duration of days significantly differs across phases (χ^2=21.08, df=3, *P*=<0.001).

Table 12. Duration of hospitalization in association with different phases of COVID-19.

Phase	N	Mean	SD	Min	Max	Median	*P*
1	600	13.67	12.99	0	80	9	
2	197	10.99	9.81	0	65	8	
3	298	12.83	12.99	0	83	9	<0.001
4	335	9.82	9.74	0	54	6	
Total	1430	12.22	11.99	0	83	8	

Data is represented as absolute numbers in days.

4.5.1. Comparison of duration of hospitalization between SCU and ICU cases

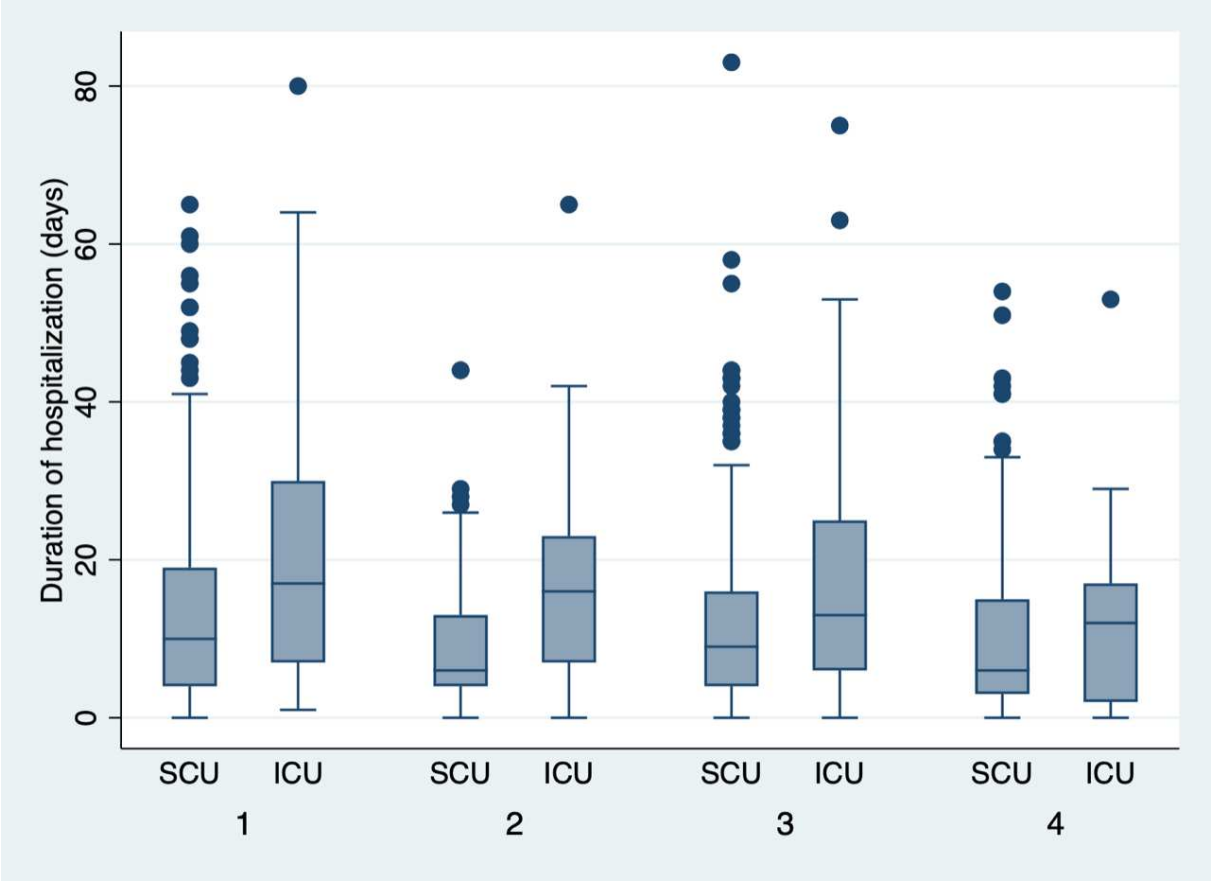
Differences in duration statistics for the two hospitalization categories (SCU and ICU) across different phases are analyzed, as indicated in Figure 3.

For cases hospitalized in standard care units (SCU), the mean duration of stay in Phase 1 is 13.41 ± 12.05 days. The minimum duration is 0 days, and the maximum duration 65 days. The median duration is indicated by p50, accounting for 10 days. Phase 2 shows the lowest mean duration with 9.1 ± 7.9 days and a minimum duration of 0 days. As maximum duration 44 days are observed, with a median duration of 6 days in Phase 2. Phase 3 follows, with the second-highest mean duration of 12.37 ± 12.37 days. Minimum and maximum duration are 0 and 83 days, respectively. Median duration is 9 days. In Phase 4 the mean duration of stay is 10.27 days. Minimum and maximum duration of stay include 0 and 54 days, respectively. Median duration is 6 days (Figure 3). There is a statistically significant difference in the distribution of hospitalization duration across the phases for SCU patients, as indicated by the Kruskal-Wallis test (χ^2=18.55, df=3, *P*=<0.001).

Regarding cases hospitalized in the ICU, a mean duration of 20.29 ± 16.01 days is observed in Phase 1. Minimum duration of stay is 1 day and 80 days observed as maximum duration. The median duration is 17 days. In Phase 2, the mean duration of stay is 16.98 ± 12.28 days. 0 days and 65 days account for the minimum and maxium duration of stay, respectively. The median duration is 16 days. 17.69 ± 17.74 days are observed as mean duration in Phase 3. The minimum duration is 0 days and the maximum duration is 75 days. The median duration is 13 days. ICU cases in Phase 4 show a mean duration of 13.52 ± 12.89 days. Similar to the other phases, mimium duration is 0 days and maximum duration is 53 days. Median duration is 12 days (Figure 3). As statistical analysis, the Kruskal-Wallis test is used, which suggests no stasticially significant difference in the distribution of duration of hospitalization across the phases for ICU cases (χ^2=4.72, df=3, *P*=0.194).

In terms of overall comparison, the mean duration for cases hospitalized in the ICU is generally higher than for SCU cases across all phases. The standard deviation (SD) also tends to be higher for ICU cases, indicating a greater variability in duration. The maximum duration is similar for both ICU categories, but there are cases of longer duration in cases hospitalized in the ICU. Higher variability is seen in across phases for the median duration of stay, with some phases showing higher medians for ICU cases compared to SCU cases. As implied by the Kruskal-Wallis test, the chi-squared value for SCU cases (χ^2=18.55) is higher than the value for ICU cases (χ^2=4.72), indicating a stronger evidence of a difference in durations across phases for SCU cases compared to ICU cases.

Figure 3. Comparison of duration of hospitalization between SCU and ICU cases.



5. DISCUSSION

This retrospective study aimed to investigate potential differences of different SARS-CoV-2 variants regarding their presence of symptoms and severity. Multiple variables were analyzed, including age, gender, presence of symptoms, immunization status, oxygen requirements, duration of hospitalization and outcome.

The demographic analysis of study population yielded several results. A total of 1430 cases has been included, with a total percentage of 51.1% males and 48.9% females, indicating that males took a slightly larger percentage in the distribution of genders. This is already indicated by other studies (72), but otherwise our study showed that there is no significant correlation with gender and phase. On the other hand, it showed a statistically significant association between the age and phase. In general, the median age which was tested positive for SARS-CoV-2 and subsequently hospitalized, was 74 years. A closer analysis of the age in correlation with gender yielded statistically significant results, with a median age in males of 73 years and in females of 76 years. Women therefore tend to be slightly older when hospitalized, in comparison to males. It is questionable, if these factors may be influenced by demographic characteristics of the population in Coburg, when compared to other results.

Regarding the presence of symptoms at admission, we could see a significant correlation between the presence of symptoms and phases. The Omikron variant, as the major variant of concern in Phase 4, showed with 60.87% the highest percentage of asymptomatic presentations, compared to all other phases. It has to be evaluated, if this observation is directly connected to the variant itself or if the presence of higher numbers of immunized individuals may influence the presence of symptoms, since vaccinations prevent and decrease symptomatic infections and their severity (54). We furthermore investigated, if the presence of symptoms may correlate in males and females with a certain age. For asymptomatic males, the median age is 73 years and for symptomatic males 72.5 years. Nonetheless, we could not define a statistic significance for the correlation of symptoms with a certain age in males. In contrast, a statistically significant difference in age between asymptomatic and symptomatic females could be detected. Asymptomatic females tend to be older with a median age of 81, whereas symptomatic females had a median age of 75. This differs from the results of studies, which usually indicate that a higher age corresponds with more severe disease courses and asymptomatic infections usually being more common in young and middle-aged individuals (37,73).

As indicated by the aims of our study, we investigated the potential difference in severity of different SARS-CoV-2 variants, by analyzing certain outcomes. In the timeframe of March 2020 and March 2022, a total of 270 deaths occurred (18.8%). The SARS-CoV-2 Wild type, as major variant of Phase 1, showed the highest percentage of death (23%), followed by

the Delta variant, or Phase 3 (21.48%). This may be explained by increased transmissibility of the Delta variant in comparison to the Alpha variant, which was the dominant variant in Phase 2. Additionally, the Delta variant is associated with increased rates of hospitalization, increased intensive care requirements and increased mortality, indicating a higher virulence of this variant, which is reflected in our study regarding the mortality (54). In contrast, Phase 4 shows the lowest percentage of death (11.04%). Again, further studies have to evaluate, if the decreased mortality in Phase 4 is directly related to the Omikron variant or if increased numbers of immunized individuals may have some influence. In addition, it must be noted, that therapeutic options have evolved greatly from Phase 1 to Phase 4, now including antiviral therapeutic agents and neutralizing monoclonal antibodies, which improved disease outcome.

Hospitalized individuals were furthermore analyzed regarding their form of inpatient care and subsequently subdivided into cases requiring treatment in the intensive care and those being treated on standard care units. Again, we could define a significant association between phases and form of inpatient care. Phase 2 showed the highest percentage of cases hospitalized in the ICU, whereas Phase 4 again had the lowest percentage of cases. This correlates with certain studies, which indicate for the Omikron variant a significant lower incidence of hospitalization and less severe disease courses (54). In addition to the form of hospitalization, the outcome of inpatient care was analyzed as well. A significant relationship between ICU/SCU status and outcome was determined. The majority of individuals hospitalized on SCU were discharged home (66.38%) and only 137 cases died (12.94%). In contrast, the majority of individuals hospitalized at the ICU died (51.42%) and only 51 cases were discharged home (24.06%). Additionally, if ICU patients were discharged, they were more often discharged into specialized care facilities, including ventilator rehabilitation facilities, compared to SCU patients. In comparison with other studies, in which mortality of ICU patients ranged from 28.3% - 35.5%, mortality of ICU patients hospitalized in the REGIOMED hospital in Coburg is increased (74,75). It has to be noted, that external ICU patients were transferred to the REGIOMED hospital in Coburg, which may lead to a certain influence on mortality rates. Still, a more detailed analysis of ICU patients' characteristics, including comorbidities and risk factors, would be necessary in order to evaluate the mortality rates further. In addition, in comparison to other countries, more patients with a poorer prognosis were admitted to the ICU due to the regulation of the German health care system leading to higher ICU mortality.

In order to further analyze the outcome of patients infected with SARS-CoV-2 the immunization status was included, which yielded highly significant results regarding the relationship between outcome and immunization status. For non-immunized individuals, an

increase in mortality (23.15%) and a decrease in home-discharge rates (50.80%) could be observed. In contrast, the higher the immunization rate, the lower the mortality (15.69%, 14.79% and 6.41% respectively). This correlates with the literature, which suggests that with increasing numbers of immunization, especially in individuals who received three vaccinations, severe and lethal disease courses are significantly reduced (54).

When correlating the number of in-hospital deaths in immunized and non-immunized case with their corresponding phase, several observations could be made: in Phase 1 a statistically significant association with the status of immunization could be made. Non-immunized individuals showed a higher mortality (24.75%) compared to immunized individuals. Still, several factors may have influenced the results at this point. Firstly, in Phase 1 vaccines were mostly not available yet, since the first vaccine was released in December 2020, and it still took several weeks until it was available for the majority of the population (76). Secondly, data sets for double-immunized individuals are not available, which may bias the results. For Phase 2 and 3 we could not define a significance for the status of immunization. Additionally, in contrast to the literature, non-vaccinated individuals had a lower mortality (15.62%) compared to single-immunized individuals (18.18%) and even to individuals immunized three times or more (15.79%) in Phase 2 and Phase 3 (18.48% vs. 19.17% and 28.36% respectively) (54). For Phase 4 we could define a statistic significance again, with non-immunized individuals showing the highest mortality rates (20.69%).

Regarding the oxygen requirements in hospitalized individuals in correlation with phases, we could determine a significant association. Individuals in Phase 4 were the least likely to require oxygen therapy (70.52%), whereas in Phase 2 the highest percentage of individuals requiring mechanical ventilation could be observed (17.11%). This correlates with the previous findings of Phase 2, which had the highest percentage of individuals hospitalized at the ICU and highest percentage of symptomatic cases, and Phase 4 with the lowest number of ICU cases and the majority of cases being asymptomatic. It would require further evaluation, why the Alpha variant, the major VOC in Phase 2, shows the highest number of individuals not only requiring mechanical ventilation, but also having the highest percentage of symptomatic and ICU cases, when the Delta variant in Phase 3 is regarded to be more virulent and with a higher transmissibility compared to the Alpha variant (54).

General duration of hospitalization was noted and yielded significant results. When analyzing the duration of hospitalization for each phase, individuals in Phase 4 had the lowest mean duration in general (9.815 days) with a median of 6 days, which matches to its previous findings. In contrast, Phase 1 had the highest mean duration of hospitalization (13.67 days). A

more detailed analysis of the duration of hospitalization in SCU and ICU cases yielded for SCU cases significant results. In general, SCU cases show a shorter mean duration of hospitalization compared to ICU patients, with a mean duration ranging from 9 days in Phase 2 to 13.412 days in Phase 1. Despite the lack of statistical significance for ICU cases, mean duration was in general longer, ranging from 13.526 days in Phase 4 to 20.298 days in Phase 1. Again, Phase 4 shows improved outcomes in comparison to Phase 1-3.

In summary, we could prove with this study multiple significant differences in disease severity and symptoms across different phases of COVID-19 but also have to consider the retrospective nature of this study, which leads to several limitations. Firstly, documentation of patient data was often limited, especially the vaccination status was often not accessible. Furthermore, definition of the vaccination status differed over time, with "fully vaccinated" individuals being vaccinated two times, later on three vaccinations were required to be fully vaccinated. Additionally, vaccinations were not fully available for the whole population, but certain groups were prioritized in the beginning. Therefore, especially in Phase 1, individuals were defined up until March 2021 as non-immunized. The records did also not take in account the type of vaccine and their varying effectiveness against different SARS-CoV-2 variants. Secondly, retrospective determination of symptoms resulted in difficulties regarding their definition since it was not always clear if the symptoms an individual presented with were directly related to an infection with SARS-CoV-2 itself. Regarding the documentation of ICU cases and their duration of hospitalization, in individuals which were transferred from external intensive care units to the REGIOMED hospital in Coburg there was a lack of documentation of their duration of hospitalization. Subsequently, the documentation of number of deaths also includes non-Covid-19-related deaths and only focusses on in-hospital deaths. The outcome of discharged patients was not noted. Possible bias also results from the classification and allocation of individuals to the four COVID-19 phases and their respective dominant SARS-CoV-2 variant based on the timeframe provided by the Robert Koch Institute. Since no genomic sequencing of RT-PCR results was performed, it may result in an inadequate assignment of individuals to different SARS-CoV-2 variants, especially due to the fluid transitions in between variants.

Despite all the limitations, there are multiple valuable findings regarding the SARS-CoV-2 variants and their possible differences. Still, further research is required in order to enable a detailed elaboration of this topic.

6. CONCLUSION

Following conclusions can be drawn from this study:

1. The different SARS-CoV-2 variants differ in their presentation of symptoms. The Omikron variant of Phase 4 shows the highest number of asymptomatic cases compared to all other variants.
2. There is a difference in disease severity across the SARS-CoV-2 variants.
3. The Wild type, as major variant of concern in Phase 1, shows the highest mean duration of hospitalization and the highest number of deaths.
4. The Alpha variant of Phase 2 has the highest number of ICU cases and the highest number of individuals requiring oxygen therapy and mechanical ventilation.
5. The Delta variant of Phase 3 shows the second highest mortality.
6. The Omikron variant of Phase 4, compared to all other variants, shows the least severe disease course. It is the variant with the lowest mortality, lowest rates of individuals admitted to the ICU, highest percentage of cases not requiring oxygen and the lowest mean duration of hospitalization.

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8. SUMMARY

Objectives:

The aim of our study was to investigate if there is a difference in the presence of symptoms in different phases of COVID-19. Additionally, we explored potential differences in SARS-CoV-2 variants regarding their disease severity.

Material and Methods:

The study was designed as a retrospective cohort study, conducted at the REGIOMED Hospital in Coburg, Bavaria, Germany. Patients that had a positive SARS-CoV-2 PCR-test result and were hospitalized at the REGIOMED hospital in Coburg from 03/2020 until 03/2022 were included. Based on data delivered from the Robert Koch Institute, patients are divided into respective study groups depending on the prevalent SARS-CoV-2 variant at the time of their positive PCR-result.

Results:

The study presented significant differences in the age distribution across different phases, as well as differences in age between males and females, but no significant gender distribution across phases. A significant difference could also be seen in the presence of symptoms across different phases. The average age in asymptomatic males was 73 years and in symptomatic males 72.5 years, but with no significant correlation. In contrast, significant results were seen in females. A significant relationship between outcome and phase could be proven, with Phase 1 showing the highest mortality. Phase 2 shows the highest percentage of cases admitted to the ICU, indicating a significant association between phases and ICU status, which could also be proven for the association between outcome and ICU status. An analysis of the outcome in correlation with immunization status showed significantly that nonimmunized individuals have an increased mortality and lower discharge rates compared to immunized individuals. The mortality in immunized and non-immunized individuals also correlates with Phase 1 and 4 but is insignificant for Phase 2 and 3. Oxygen requirements differed across the Phases, yielding significant results. The general duration of hospitalization differed significantly across phases, with Phase 1 having the highest mean duration of hospitalization, whereas Phase 4 the shortest.

Conclusion:

The SARS-CoV-2 variants differ in their presentation of symptoms as well as in disease severity, presenting differences in the duration and type of hospitalization, oxygen requirements and outcome.

9. CROATIAN SUMMARY

Ciljevi:
Cilj našeg istraživanja bio je ispitati postoji li razlika u prisutnosti simptoma u različitim fazama COVID-19. Dodatno smo istražili potencijalne razlike u težini bolesti između varijanti SARS-CoV-2.

Materijali i metode:
Studija je osmišljena kao retrospektivna kohortna studija, provedena u bolnici REGIOMED u Coburgu, Bavarska, Njemačka. Uključeni su pacijenti koji su imali pozitivan rezultat PCR-testa na SARS-CoV-2 i koji su bili hospitalizirani u bolnici REGIOMED u Coburgu od 03/2020. do 03/2022. godine. Na temelju podataka dobivenih od Robert Koch instituta, pacijenti su podijeljeni u odgovarajuće studijske skupine ovisno o prevladavajućoj varijanti SARS-CoV-2 u vrijeme pozitivnog PCR rezultata.

Rezultati:
Studija je pokazala značajne razlike u dobnom rasporedu tijekom različitih faza, kao i razlike u dobi između muškaraca i žena, ali nije bila značajna rodna raspodjela tijekom faza. Također se vidjela značajna razlika u prisutnosti simptoma tijekom različitih faza. Prosječna dob kod asymptomatskih muškaraca iznosila je 73 godine, a kod simptomatskih muškaraca 72,5 godina, ali bez značajne korelacije. S druge strane, značajni rezultati su bili vidljivi kod žena. Dokazan je značajan odnos između ishoda i faze, pri čemu je Faza 1 pokazala najvišu smrtnost. Faza 2 pokazuje najviši postotak slučajeva primljenih u jedinicu intenzivnog liječenja, što ukazuje na značajnu vezu između faza i statusa JIL-a, što se također moglo dokazati i za vezu između ishoda i statusa JIL-a. Analiza ishoda u vezi s imunizacijskim statusom pokazala je značajno da necijepljene osobe imaju povećanu smrtnost i niže stope otpusta u usporedbi s cijepljenim osobama. Smrtnost kod cijepljenih i necijepljenih osoba također korelira s Fazom 1 i 4, ali nije značajna za Faze 2 i 3. Potrebe za kisikom razlikovale su se tijekom Faza, rezultirajući značajnim rezultatima. Općenito trajanje hospitalizacije značajno se razlikovalo između faza, pri čemu je Faza 1 imala najduže prosječno trajanje hospitalizacije, dok je Faza 4 imala najkraće.

Zaključak:
Varijante SARS-CoV-2 razlikuju se u prikazu simptoma, kao i u težini bolesti, prikazujući razlike u trajanju i vrsti hospitalizacije, potrebama za kisikom i ishodu.

10. CURRICULUM VITAE

Personal data

Name	Johanna Huntemann
Date of and place of birth	05.09.1998 in Lüdenscheid, Germany
Address	Ketschendorfer Str. 25 96450 Coburg, Germany

Education

10.2017 – 10.2023	**University of Split, School of Medicine** Medical Studies in English 2nd and 3rd year Co-leader ISA event management 6th year student representative
08.2008 – 06.2016	**Bergstadt-Gymnasium Lüdenscheid** General qualification for university entrance Advanced courses: Biology, Latin

Internships

12.09.2022 – 23.10.2022	**REGIOMED Klinikum Coburg** Accident and emergency unit
12.07.2021 – 08.08.2021	**REGIOMED Klinikum Coburg** Accident and emergency unit
11.2016 – 02.2017	**Klinikum Lüdenscheid** Visceral surgery Nursing Internship