DETERMINATION OF TOTAL IMMUNOGLOBULIN G AND IMMUNOGLOBULIN G SUBCLASSES RESPONSE AGAINST SARS-COV-2 OMICRON VARIANT IN PFIZER AND SINOVAC VACCINATED SERUM SAMPLES

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UNIVERSITI SAINS MALAYSIA

2025

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by

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Dissertation submitted in partial fulfillment of the requirements for the Degree of Bachelor of Health Science (Honours) (Biomedicine)

January 2025

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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Date: 27 January 2025

ACKNOWLEDGEMENTS

I begin with heartfelt gratitude to God, whose blessings, guidance, and strength have enabled me to persevere and complete this research project. First and foremost, I wish to express my deepest appreciation to my supervisor, Assoc. Prof. Dr. Rapeah Suppian, for her steadfast support, expert guidance, and invaluable insights throughout this research journey. Her patience, encouragement, and dedication have been instrumental in overcoming challenges and refining my understanding of this field. I am profoundly grateful to the research assistants and postgraduate students, especially Dr. Aini, Ms. Atiqa, and Ms. Afiqah, for their generosity in sharing their expertise, their unwavering support, and their invaluable assistance during the experimental and data analysis phases. My sincere thanks also extend to the staff at the School of Health Sciences, Universiti Sains Malaysia, for granting me access to the facilities and resources necessary to carry out this research. I would like to extend special thanks to my close friends—Laavanya A/P Karunanithi, Sharran A/L Selvapaandian, Danesh A/L Chandiran, Dharshiga A/P Ravi, Patricia Jane A/P Wilfred Martin, Kian Sedrick Soosay, Nimallan A/L Ahrasan, Yugaashini A/P Partheeban—for their steadfast encouragement, kindness, and unwavering friendship. Your presence has been a source of constant motivation and joy throughout my academic journey. Above all, I am profoundly indebted to my family—my parents, Mr. Ravi A/L Chinnappan and Mrs. T. Komate A/P Tambai @ Muniandy, my siblings; Yuvanraj A/L Ravi, Sachiin A/L Ravi, and my extended family—for their unconditional love, sacrifices, and encouragement. Their unwavering belief in my potential has been the foundation of my success. This thesis is a reflection of the collective efforts, support, and inspiration of all the remarkable individuals who have contributed to this journey. To all of you, I offer my heartfelt gratitude.

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LIST OF SYMBOLS AND ABBREVIATIONS

^{o}C	Degree Celsius
µL	Microliter
mL	Milliliter
%	Percentage
nm	nanometer
ACE2	Angiotensin-converting enzyme-2
ADCC	Antibody-dependent cellular cytotoxicity
ANOVA	Analysis of variance
ARDS	Acute respiratory distress syndrome
CD4+	T-helper cells
CD8+	Cytotoxic T cells
CI	Confidence interval
COVID-19	Coronavirus disease 2019
ECD	Extracellular domain
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FcRs	Fc Receptors
HRP	Horseradish peroxidase
IGA	Immunoglobulin A
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1

IgG2	Immunoglobulin G2
IgG3	Immunoglobulin G3
IgG4	Immunoglobulin G4
IGM	Immunoglobulin M
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
kDa	Kilodalton
RNA	Ribonuclei acid
mRNA	Messenger-RNA
OD	Optical density
PBS	Phosphate buffered saline
PBST	1x Phosphate buffered saline with Tween-20
PCR	Polymerase chain reaction
PPV	Pusat Pemberian Vaksin
PUI	People under investigation
RBD	Receptor-binding domain
RT	Room temperature
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SE	Standard error

TMB	3,3’,5,5’ - tetramethylbenzidine
USM	Universiti Sains Malaysia
WHO	World Health Organisation

PENENTUAN TAHAP IMMUNOGLOBULIN G (IgG) TOTAL DAN GERAK BALAS SUBKELAS IgG TERHADAP VARIAN OMICRON SARS-COV-2 DALAM SAMPEL SERUM VAKSIN PFIZER DAN SINOVAC

ABSTRAK

Pandemik COVID-19, yang disebabkan oleh virus SARS-CoV-2 yang sangat mudah menular, terus menjadi ancaman utama kepada kesihatan global. Kemunculan varian baharu seperti Omicron telah menimbulkan kebimbangan terhadap kebolehan virus mengelak sistem imun, kemerosotan imuniti dari semasa ke semasa, serta keberkesanan jangka panjang vaksin COVID-19. Walaupun vaksinasi kekal sebagai langkah paling berkesan dalam mengurangkan risiko jangkitan teruk dan penularan, variasi dalam gerak balas imun yang dicetuskan oleh platform vaksin yang berbeza memerlukan penyelidikan yang lebih mendalam. Oleh itu, kajian ini dijalankan untuk menilai gerak balas imun humoral dengan meneliti tahap keseluruhan Immunoglobulin G (IgG) serta subkelasnya, IgG1 dan IgG4, dalam kalangan individu yang menerima vaksin Pfizer-BioNTech (BNT162b2) dan Sinovac (CoronaVac). Kajian ini melibatkan 14 peserta, dengan tujuh daripadanya menerima vaksin Pfizer dan tujuh lagi menerima vaksin Sinovac. Sampel serum dikumpulkan pada enam titik masa utama, iaitu sebelum vaksinasi, selepas dos pertama, dua minggu selepas dos kedua, serta selepas dos penggalak pada minggu kedua, ke-26, dan ke-52. Teknik ujian enzim imunosorben berkait (ELISA) digunakan bagi mengukur gerak balas IgG spesifik terhadap protein pepaku SARS-CoV-2. Keputusan kajian menunjukkan peningkatan ketara dalam jumlah IgG bagi kedua-dua kumpulan vaksin sepanjang tempoh kajian ($p < 0.0001$). Penerima vaksin Sinovac mencatatkan tahap IgG keseluruhan yang lebih tinggi (min jangkaan: 1.984) berbanding penerima vaksin Pfizer (min jangkaan: 1.442). Walau bagaimanapun, tahap IgG1 didapati hampir setara antara kedua-dua kumpulan sepanjang tempoh kajian

($p > 0.05$), dengan vaksin Sinovac menunjukkan peningkatan sementara pada minggu kedua dan ke-26 selepas dos penggalak. Sebaliknya, tahap IgG4 meningkat dengan ketara dalam kalangan penerima vaksin Pfizer pada peringkat dos penggalak yang lebih lewat (minggu ke-26 dan ke-52, $p < 0.05$), manakala penerima Sinovac menunjukkan gerak balas IgG4 yang lebih sederhana. Hasil kajian ini mencadangkan bahawa vaksin Sinovac berupaya merangsang gerak balas IgG keseluruhan yang lebih kuat pada peringkat awal, manakala vaksin Pfizer cenderung mencetuskan peningkatan IgG4 yang lebih ketara selepas dos penggalak. Pola pengagihan subkelas IgG yang berbeza ini berpotensi mempengaruhi daya tahan imuniti jangka panjang serta mekanisme pengawalan gerak balas imun selepas vaksinasi. Kefahaman yang lebih mendalam terhadap perbezaan gerak balas imun ini amat penting dalam memperhalusi strategi dos penggalak, memperkukuh dasar vaksinasi, serta memastikan perlindungan yang lebih berkesan terhadap varian baharu SARS-CoV-2.

DETERMINATION OF TOTAL IMMUNOGLOBULIN G (IgG) LEVELS AND IgG SUBCLASSES RESPONSE AGAINST SARS-COV-2 OMICRON VARIANT IN PFIZER AND SINOVAC VACCINATED SERUM SAMPLES

ABSTRACT

The COVID-19 pandemic, caused by the highly transmissible SARS-CoV-2 virus, continues to pose significant global health challenges. The emergence of variants such as Omicron has raised concerns regarding immune escape, waning immunity, and the long-term effectiveness of COVID-19 vaccines. While vaccination remains the most effective strategy in mitigating severe disease and transmission, differences in immune responses elicited by various vaccine platforms necessitate further investigation. This study aimed to evaluate the humoral immune response, focusing on total Immunoglobulin G (IgG) levels and IgG subclasses (IgG1 and IgG4), in individuals vaccinated with Pfizer-BioNTech (BNT162b2) and Sinovac (CoronaVac) vaccines. A total of 14 participants were recruited, with seven receiving Pfizer and seven receiving Sinovac. Serum samples were collected at six critical time points: pre-vaccination, post-first dose, two weeks after the second dose, and post-booster doses at two, 26, and 52 weeks. An indirect enzyme-linked immunosorbent assay (ELISA) was employed to quantify spike-specific IgG responses. The results demonstrated a significant increase in total IgG levels over time in both vaccine groups ($p < 0.0001$). Sinovac recipients exhibited higher total IgG levels (predicted mean: 1.984) compared to Pfizer recipients (predicted mean: 1.442). IgG1 levels remained comparable between both groups across all time points ($p > 0.05$), with Sinovac showing a transiently higher IgG1 response at two- and 26-weeks post-booster. In contrast, IgG4 levels significantly increased in Pfizer recipients at later booster time points (26 and 52 weeks, $p < 0.05$), whereas

Sinovac elicited a less pronounced IgG4 response. These findings suggest that Sinovac induces a stronger early total IgG response, while Pfizer leads to a more pronounced IgG4 response after booster doses. The distinct patterns of IgG subclass distribution may influence long-term immunity and immune regulation following vaccination. Understanding these differential immune responses is essential for optimizing booster strategies, refining vaccine policies, and ensuring prolonged protection against emerging SARS-CoV-2 variants.

CHAPTER ONE: INTRODUCTION

1.1 Research Background

The Coronavirus Disease 2019 (COVID-19) is a severe respiratory illness caused by the highly infectious virus, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). Initially identified in Wuhan, China, in December 2019, COVID-19 rapidly spread across the globe, prompting the World Health Organization (WHO) to declare it a pandemic on March 11, 2020. The virus's swift transmission and widespread impact have resulted in significant global health, social, and economic challenges. As of August 16, 2023, there have been 769,806,130 confirmed cases and 6,955,497 deaths worldwide, with Malaysia reporting 5,125,089 cases and 37,187 deaths (Datadot, 2024). COVID-19's severity varies, ranging from mild or asymptomatic cases to critical illness, particularly in individuals with underlying health conditions such as cardiovascular disease, diabetes, or respiratory disorders. The virus can cause complications like pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure, which may require intensive care or result in death (Cascella, 2023).

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA (ssRNA) virus belonging to the betacoronavirus genus, similar to other viruses in the coronavirus family, such as SARS-CoV and MERS-CoV. The virus has four primary structural proteins, including the spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and envelope (E) protein. Among these, the S and N proteins are the most significant in eliciting an immune response. The S protein is responsible for mediating the virus's entry into human cells by binding to the angiotensin-

converting enzyme 2 (ACE2) receptor on the surface of host cells. The S protein consists of two subunits: S1, which contains the receptor-binding domain (RBD) that facilitates viral attachment, and S2, which enables the fusion of the viral and host cell membranes, allowing the virus to enter the cell (Kakavandi et al., 2023).

The immune system recognizes SARS-CoV-2 as a foreign pathogen, triggering an immune response that involves both innate and adaptive mechanisms. The adaptive immune response includes the production of neutralizing antibodies that specifically target the viral proteins, particularly the spike protein. These antibodies consist primarily of immunoglobulin (Ig) M, IgA, and IgG isotypes. IgM and IgA are produced early in the immune response, whereas IgG, which is the most abundant immunoglobulin in human serum, is produced later and provides long-lasting immunity (Boechat et al., 2021).

IgG antibodies can be subdivided into four subclasses—IgG1, IgG2, IgG3, and IgG4—each with distinct roles in immune defence. This study focuses specifically on IgG antibodies targeting the spike protein, as they are crucial for providing immunity against the virus and are commonly used as markers of past infection or vaccination.

In response to the COVID-19 pandemic, extensive public health measures have been implemented worldwide to mitigate the spread of the virus. These measures have included promoting social distancing, mask-wearing, frequent handwashing, and limiting large gatherings. Despite these efforts, the rapid pace of SARS-CoV-2 transmission has necessitated the development and distribution of vaccines.

Vaccination has emerged as one of the most effective strategies to curb the spread of the virus and reduce the severity of infections. COVID-19 vaccines work by stimulating the immune system to produce antibodies and activate T cells, creating a memory response that can recognize and fight the virus upon future exposure.

Several COVID-19 vaccines have been developed globally, including those from Sinovac, AstraZeneca, Cansino, and Pfizer. These vaccines are designed to mimic the immune response generated by natural infection with SARS-CoV-2, without causing the disease (Mayo Clinic, 2024). The rapid development and rollout of COVID-19 vaccines have played a crucial role in reducing the number of severe cases and deaths worldwide, although vaccine coverage and hesitancy remain ongoing challenges.

The Pfizer-BioNTech (BNT162b2) vaccine is an mRNA-based COVID-19 vaccine that utilizes lipid nanoparticles to deliver genetic instructions encoding the SARS-CoV-2 spike protein, thereby stimulating an immune response without causing infection. Clinical trials have demonstrated its high efficacy, with a two-dose regimen conferring 95% protection against COVID-19 in individuals aged 16 and older (Polack et al., 2020). In contrast, the Sinovac (CoronaVac) vaccine is an inactivated virus vaccine that employs chemically inactivated SARS-CoV-2 particles to trigger an immune response. A large phase 3 trial in Brazil indicated that two doses of CoronaVac, administered 14 days apart, had an efficacy of 51% against symptomatic SARS-CoV-2 infection and 100% efficacy against severe COVID-19 and hospitalization starting 14 days after the second dose (World, 2022). Both vaccines play crucial roles in global immunization efforts, with differences in

immune response dynamics influencing their long-term effectiveness and booster strategies.

In addition to vaccination, ongoing efforts include improving diagnostic methods for detecting SARS-CoV-2 infections, such as PCR testing and antigen-based tests, as well as enhancing treatments for COVID-19. Antiviral medications, monoclonal antibodies, and immune modulators have been explored to manage severe cases, and research into long COVID (persistent symptoms following initial infection) continues to evolve. Ongoing surveillance, research, and public health interventions remain essential in managing and ultimately controlling the COVID-19 pandemic (Zhou et al., 2021).

Given the differences in immune response dynamics between mRNA-based and inactivated virus vaccines, this study aimed to evaluate the humoral immune response in individuals vaccinated with Pfizer-BioNTech (BNT162b2) and Sinovac (CoronaVac). Specifically, the study focused on assessing total Immunoglobulin G (IgG) levels and IgG subclasses (IgG1 and IgG4) at multiple time points, including pre-vaccination, post-primary doses, and post-booster doses. Using an enzyme-linked immunosorbent assay (ELISA), this research sought to compare the magnitude and persistence of vaccine-induced IgG responses, providing insights into the long-term immune protection and potential differences in immune regulation between the two vaccine platforms. Understanding these differences is essential for optimizing booster strategies and informing future vaccination policies, particularly in light of emerging SARS-CoV-2 variants.

1.2 Problem statement

Despite widespread administration of the Pfizer COVID-19 vaccine, there remains a limited understanding of the long-term dynamics of total IgG and IgG subclass responses post-vaccination. This gap in knowledge poses challenges in accurately assessing population immunity and understanding the potential impacts of the Omicron variant on public health.

1.3 Objectives

1.3.1 General Objectives

To compare the effect of Omicron variant infection on total IgG and IgG subclass responses in the serum of Pfizer and Sinovac vaccine recipients.

1.3.2 Specific objectives

I. To determine the levels of total immunoglobulin G (IgG) and IgG subclasses (IgG1 and IgG4) in the serum of USM PPV recipients vaccinated with Pfizer and Sinovac.

II. To evaluate the trends in IgG, IgG1, and IgG4 levels across different post-vaccination and post-booster time points for Pfizer and Sinovac recipients.

III. To compare the differential IgG subclass responses between Pfizer and Sinovac recipients, particularly concerning booster doses and long-term immunity.

1.4 Hypothesis

1.4.1 Null hypothesis (H_0)

There will be no significant difference in the levels of total immunoglobulin G (IgG) and IgG subclasses response in human serum after vaccination with the Pfizer and Sinovac vaccine against the Omicron variant of the SARS-CoV-2, evaluating the immune response triggered by the variant.

1.4.2 Alternative hypothesis (H_A)

There will be a significant difference in the levels of total immunoglobulin G (IgG) and IgG subclasses response in human serum after vaccination with the Pfizer and Sinovac vaccine against the Omicron variant of the SARS-CoV-2, evaluating the immune response triggered by the variant.

1.5 Rational of the study

The evolution of SARS-CoV-2 and its variants continued to challenge global public health efforts, necessitating detailed studies on immune responses elicited by COVID-19 vaccines. This study aimed to determine whether serum samples collected from individuals vaccinated with the Pfizer mRNA vaccine had been exposed to SARS-CoV-2 infection from either the Wuhan or Omicron variant before vaccination or booster doses. This knowledge was vital for public health officials to make informed decisions on vaccine distribution, booster recommendations, and outbreak control, especially as new variants emerged (Barnes et al., 2023). A key focus of this study was the humoral response to the Pfizer vaccine, specifically Immunoglobulin G (IgG) and its subclasses. IgG played a major role in the body's humoral defence against SARS-CoV-2 (Oliveira-Silva et al., 2022). IgG subclasses (IgG1, IgG2, IgG3, and IgG4) exhibited unique properties, including differences in hinge length, complement activation, plasma concentration, molecular weight, and half-life. Although previous studies had primarily measured total IgG in response to SARS-CoV-2 immunization, the specific trends and roles of each IgG subclass over time remained unclear (Møller et al., 2024).

Research suggested that during acute SARS-CoV-2 infection, patients typically produced RBD-specific IgG1 and IgG3, while IgG2 and IgG4 were less prominent in serum. However, the decline of neutralizing antibodies over time in individuals infected naturally with COVID-19 raised the need for a better understanding of IgG subclass dynamics following vaccination (Nogimori et al., 2024). Monitoring the specific IgG subclasses, particularly after mRNA vaccination, could reveal trends that predicted vaccine effectiveness and the durability of the immune response.

Recent studies have noted a marked increase in IgG4 levels in individuals who had received multiple doses of mRNA vaccines. This exclusive increase in IgG4 after the third dose correlated with a poor illness prognosis when the IgG4/IgG1 ratio was high. Therefore, this study quantified total IgG and individual IgG subclasses, mainly IgG1 and IgG4, in serum samples using an indirect enzyme-linked immunosorbent assay (ELISA). The samples were assessed against the Wuhan-Hu SARS-CoV-2 spike (S1) protein to determine the response specific to the Pfizer vaccine. The findings were expected to provide insight into the long-term efficacy of homologous Pfizer vaccination (Manirambona et al., 2024). By identifying which IgG subclasses were predominantly induced by vaccination, this study contributed to understanding the necessity of booster vaccines and improving strategies for SARS-CoV-2 prevention in the face of a changing viral landscape.

Research suggests that during acute SARS-CoV-2 infection, patients typically produce RBD-specific IgG1 and IgG3, while IgG2 and IgG4 are less prominent in serum. However, the decline of neutralizing antibodies over time in individuals infected naturally with COVID-19 raises the need for a better understanding of IgG subclass dynamics following vaccination (Nogimori et al., 2024). Monitoring the specific IgG subclasses, particularly after mRNA vaccination, may reveal trends that predict vaccine effectiveness and durability of immune response.

CHAPTER TWO: LITERATURE REVIEW

2.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

2.1.1 Epidemiology of SARS-CoV-2

The COVID-19 pandemic, officially known as Coronavirus Disease 2019, represents a monumental public health crisis caused by the highly infectious SARS-CoV-2 virus. Originating from a mysterious outbreak of pneumonia-like cases, the disease was first detected in Wuhan, Hubei Province, China, in December 2019. Within mere months, COVID-19 ignited a global health emergency, spreading at an unprecedented rate. By March 11, 2020, the World Health Organization (WHO) had declared COVID-19 a pandemic due to its alarming transmissibility and the staggering number of severe illnesses and fatalities that ensued worldwide (Pollard et al., 2020).

As of the latest WHO reports in 2024, over 770 million confirmed COVID-19 cases and more than 7 million deaths have been documented globally, underscoring the virus's deadly reach and long-lasting impact. In Malaysia alone, over 5.1 million cases and approximately 37,000 deaths have been recorded since the pandemic's start in early 2020.

Malaysia reported its first COVID-19 case on January 25, 2020, when a traveler from China tested positive. From then on, the country was thrust into a relentless battle to contain the virus, challenging its public health infrastructure in unprecedented ways. The initial cases in Malaysia were imported, reaching 22 by mid-February. The first wave saw cases among people under investigation (PUIs), close contacts, and two Malaysians repatriated from Wuhan, China. All infected individuals in this phase were

successfully treated. The second wave, beginning on February 27, 2020, saw cases rise dramatically, involving individuals with travel histories to countries experiencing outbreaks, such as China, Japan, Italy, and Australia. By March 10, Malaysia recorded 129 cases among PUIs, close contacts, and individuals evacuated during humanitarian efforts (Health, 2024).

One of the most notable early clusters was linked to Malaysia's 26th COVID-19 patient, who had recently returned from Shanghai and, unknowingly infected, participated in numerous gatherings, spreading the virus to over 120 others. This highly contagious cluster highlighted the virus's capacity to spread rapidly through community interactions. Investigations by the Institute for Medical Research identified a potential mutation in the viral strain from this patient, suggesting a spike mutation possibly enhancing the virus's infectivity and transmissibility (Volz et al., 2020). SARS-CoV-2 believed to have originated from bat-related genetic mutations, continued to evolve. In Malaysia, strain B, predominant in East Asia, displayed unique mutations compared to strains A and C, complicating vaccine development efforts. The virus’s relentless mutations have presented an ongoing challenge to pharmaceutical advancements, underscoring the need for adaptive vaccine strategies against emerging variants.

2.1.2 Signs and Symptoms

Due to its primary affinity for the respiratory system, COVID-19 generally presents in symptomatic patients with a range of pneumonia-like signs, including fever, fatigue, diarrhoea, sore throat, dry cough, pharyngitis, muscle pain, and difficulty breathing, typically appearing 2 to 14 days after exposure as referred in Figure 2.1. Notably, a widespread and distinguishing symptom is the loss or alteration of smell and taste,

which often marks COVID-19 infections. In some cases, respiratory symptoms may intensify in the second week, even after the fever subsides. COVID-19 symptoms can progress into a systemic infection, compromising multiple organ systems—such as cardiovascular, respiratory, gastrointestinal, neurological, haematological, and immune systems. The virus's potential complications, although affecting a minority, are severe and diverse, including myocardial infarction, acute respiratory distress, renal failure, thrombotic microangiopathies, seizures, antiphospholipid syndrome, and refractory thrombosis. However, the mechanisms underlying why only a fraction of SARS-CoV-2 patients suffer extensive organ damage remain elusive (Bourgonje et al., 2024).

Research reveals that ACE2 activity—a key receptor for SARS-CoV-2—is elevated in children aged 4 to 13, tapering off in adolescence. This variation might explain the higher prevalence of pulmonary fibrosis in young children, due to the virus’s high affinity for the ACE2 receptor's spike protein. Additionally, children's distinct CD4+ and CD8+ T cell populations contrast sharply with adults, resulting in a more favourable prognosis and lower mortality rates in severe pediatric cases (Cicco et al., 2021).

The severity of COVID-19 displays a dramatic spectrum, varying widely across individuals from asymptomatic cases to critical illnesses that demand hospitalization, ICU admission, or even life-saving interventions like supplemental oxygen and mechanical ventilation. This severity is largely influenced by host factors and other risk determinants, including age, comorbidities, and immune status. Older adults and those with chronic health conditions—such as diabetes, hypertension, obesity, asthma, recipients of organ or stem cell transplants, cancer patients, and individuals with chronic kidney or liver disease—remain especially vulnerable to COVID-19. These individuals

face a heightened risk of moderate to severe illness, increased susceptibility to infection, more intense clinical symptoms, and significantly elevated hospitalization rates.

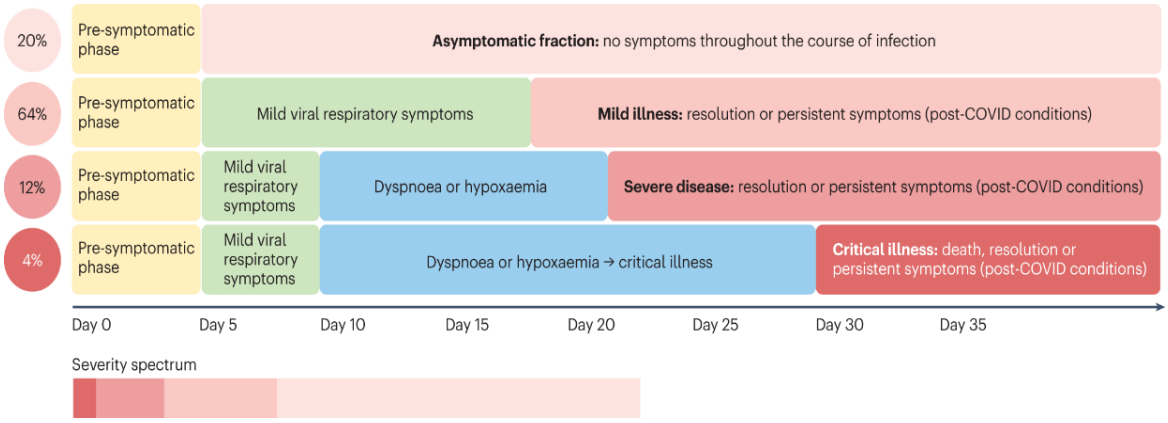

Figure 2.1: Progression of COVID-19 symptoms and stages (Meyerowitz et al., 2023)

As of 2024, those aged 65 and older still represent over 81% of COVID-19 fatalities, with death rates among this group approximately 97 times higher than those aged 18-29. In addition, individuals who are unvaccinated or who have suffered multisystem inflammatory syndrome (MIS) during or after infection are at heightened risk of enduring a range of long-term complications during recovery, known collectively as post-acute COVID-19 or "long COVID." This condition emerges around four weeks after the initial infection, often persisting indefinitely and exerting lasting effects on major organ systems like the lungs, heart, and brain.

Persistent symptoms associated with long COVID often include overwhelming fatigue unrelieved by rest, shortness of breath, and chronic coughing. These respiratory effects are primarily due to abnormal accumulations of fibroblasts and myofibroblasts, unregulated matrix metalloproteinase activity, and severe cytokine storms that initiate and accelerate pulmonary fibrosis, potentially culminating in acute respiratory distress

syndrome (ARDS). Additionally, the systemic inflammatory response associated with COVID-19 heightens the risk of cardiac complications, such as heart failure, heart attacks, and arrhythmias, driven by myocardial inflammation, activation of coagulation pathways, and damage to blood vessel linings (Shreeya et al., 2024).

Neurological complications have also proven significant post-COVID, as the virus can cause structural changes in the brain, notably a reduction in grey matter thickness, which may lead to symptoms like brain fog, cognitive impairments, and neurovegetative disturbances. Given the virus's association with multisystem organ dysfunction, especially among at-risk populations, COVID-19 remains a serious and potentially life-threatening illness, underscoring the urgent need for comprehensive prevention and long-term care strategies.

2.1.3 SARS-CoV-2 Structure

SARS-CoV-2, the causative agent of COVID-19, belongs to the Betacoronavirus genus within the Coronaviridae family. Genetically, it exhibits approximately 79% sequence similarity with SARS-CoV and around 50% with MERS-CoV. These three pathogenic coronaviruses, which have emerged since the turn of the 21st century, have crossed species barriers to cause severe respiratory infections in humans, often resulting in high mortality rates (Yu et al., 2023). SARS-CoV-2 is an enveloped virus with a helical nucleocapsid comprised of nucleoproteins (N) bound to a positive-sense single-stranded RNA (ssRNA) genome. This genome encodes four critical structural proteins that drive viral replication and pathogenicity: the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, each embedded in the viral envelope, which under electron microscopy resembles a crown—a hallmark of coronaviruses. The spike protein, presented as a full-length trimer, is fundamental to the virus’s entry into host cells,

orchestrating COVID-19 pathogenesis (Jackson et al., 2021).

The M protein, existing as a 25 to 30 kDa dimer across several virions, includes two transmembrane domains, an N-terminal ectodomain, and a C-terminal endodomain. This composition aids in shaping membrane curvature and binding with the nucleocapsid (Zhang et al., 2022). In contrast, the E protein, a smaller 8-12 kDa component, acts as an ion channel—a key factor in the pathogenicity of SARS-CoV and likely SARS-CoV-2. This transmembrane protein, with both an N-terminal ectodomain and a C-terminal endodomain, is essential for viral assembly and release (Mandala et al., 2020). The N protein is the virus's main RNA-binding component, heavily phosphorylated to strengthen its interaction with viral RNA, which binds in a "beads-on-a-string" conformation. This protein is pivotal in packaging the encapsulated genome into a helical ribonucleocapsid through associations with the M protein and nsp3, a replicase component that attaches to the replicase-transcriptase complex (RTC). Due to its abundance, the N protein serves as a valuable early diagnostic marker (Syed Nabeel-Shah et al., 2021).

The E protein, although small, performs multiple roles, including providing structural support to the viral capsid, aiding in viral assembly, and functioning as a viroporin, which enhances viral pathogenicity (Pizzato et al., 2021). Of paramount importance, however, is the S protein, the principal viral component eliciting an immune response in the host. It binds to host cell receptors, enabling viral entry independently of other components, and initiates cell signalling within human host cells. This 150 kDa N-glycosylated protein is instrumental in endoplasmic reticulum evaluation. Trimers of the S protein constitute the virus's characteristic spikes, and the S protein itself, a class I

fusion protein, is crucial for receptor binding. Host cell proteases, such as furin-like enzymes, cleave the S protein into two active domains, S1 and S2, each playing a significant role in the viral replication cycle (Pizzato et al., 2021).

The receptor-binding domain (RBD) within the S1 region of the S protein recognizes and interacts with human angiotensin-converting enzyme 2 (hACE2), facilitating viral entry. This interaction triggers structural shifts in the S2 subunit, leading to the insertion of its fusion peptide into the target cell membrane, initiating the fusion process. Studies indicate that SARS-CoV-2's high infectivity stems from mutations within the RBD of the S protein, optimizing viral attachment and entry (Jackson et al., 2021). Additionally, hACE2 is highly expressed in various organs, particularly in lung alveolar epithelial and intestinal epithelial cells, which renders these organs especially vulnerable (Lobna Al-Zaidan et al., 2021). Other tissues, including enterocytes, endothelial cells, and smooth muscle cells, also exhibit substantial ACE2 expression, expanding the potential sites for viral infection.

The RBD of the S1 subunit represents a critical target for developing diagnostics, therapies, and vaccines. The highly immunogenic RBD domain of the SARS-CoV-2 spike protein triggers robust IgG antibody responses in both acute and convalescent cases, marking it as a viable target for serological testing. This project is dedicated to detecting various spike-specific IgG subclasses—particularly spike-specific IgG1 and IgG4—as well as the total serum spike-specific IgG directed against the full-length S protein of the Wuhan-1 strain in individuals vaccinated with the Pfizer vaccine (Huang et al., 2020).

2.1.4 SARS-CoV-2 Viral Variants

SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has evolved significantly since its emergence in late 2019, leading to the identification of various viral variants. These variants are classified based on their genetic mutations and their impact on transmissibility, severity of disease, and vaccine efficacy. Among the notable variants, the original strain from Wuhan and the Omicron are particularly significant.

The initial strain of SARS-CoV-2, often referred to as the Wuhan variant or WIV04/2019, was first identified in Wuhan, China, in December 2019. This variant serves as a reference point for subsequent genetic analyses and is characterized by a relatively simple mutation profile compared to later variants. Key mutations in this strain include those in the receptor-binding domain (RBD) of the spike protein, notably N501Y, K417N, and E484K. These mutations enhance the virus's ability to bind to human ACE2 receptors, facilitating infection. The Wuhan variant has been foundational in understanding the virus's biology and used extensively in vaccine development. However, as the pandemic progressed, this variant was largely supplanted by more transmissible and virulent strains (Cascella, 2023).

On the other hand, the Omicron variant (B.1.1.529), which is primarily used in this study, first reported in South Africa in November 2021, represents a significant evolution of SARS-CoV-2. It is distinguished by an extensive array of mutations—over 30 in the spike protein alone—which contribute to its high transmissibility and potential immune evasion capabilities. Omicron is estimated to be over ten times more contagious than the original Wuhan strain. Studies indicate that it can replicate more efficiently in the upper respiratory tract compared to previous variants but shows reduced replication

efficiency in lung tissues. The Omicron variant features several critical mutations such as S371L, G339D, S477N, and N501Y. These mutations are associated with enhanced binding affinity to human cells and may contribute to their ability to evade neutralizing antibodies generated by previous infections or vaccinations. While Omicron infections tend to result in less severe disease compared to earlier variants like Delta, they still pose significant public health challenges due to their rapid spread. The variant has led to increased hospitalizations, particularly among unvaccinated populations. Since its emergence, several subvariants of Omicron have been identified (e.g., BA.1, BA.2), each exhibiting slight variations in transmissibility and immune escape potential. The BA.2 subvariant has shown dominance over BA.1 in various regions due to its enhanced spread (Silva et al., 2023).

While the Wuhan variant laid the groundwork for understanding SARS-CoV-2's biology and developing vaccines, the emergence of variants like Omicron highlights the ongoing challenges posed by viral evolution in managing COVID-19 effectively. Continuous surveillance and research are essential for adapting public health responses and vaccination strategies against these evolving threats.

2.1.5 SARS-CoV-2 Omicron Variant

The Omicron variant of SARS-CoV-2, designated B.1.1.529, was first identified in late 2021 and rapidly became the predominant strain globally, primarily due to its extraordinary transmissibility and extensive mutation profile. Omicron exhibits over 60 mutations, with approximately 37 located in the spike protein, which is crucial for viral attachment to and entry into human cells. Among these, 15 mutations are concentrated in the receptor-binding domain (RBD) of the spike protein, including key alterations such as N501Y, K417N, and E484A. These specific mutations enhance binding affinity to the human ACE2 receptor and allow the virus to evade immune responses, particularly by reducing the efficacy of neutralizing antibodies generated by both previous infections and vaccinations (Fan et al., 2022).

The significant ability of Omicron to evade immunity has led to widespread reinfections and breakthrough cases among vaccinated individuals, contributing to public health challenges worldwide. The basic reproduction number (R0) for Omicron has been estimated as high as 8.4, considerably higher than earlier strains like Delta. This high transmissibility has driven unprecedented surges in cases globally, stretching healthcare resources to their limits despite Omicron generally being associated with less severe illness (Yadav et al., 2022). The rapid spread has not only overwhelmed healthcare systems but also disrupted workforce availability, education, and economies, with many countries facing challenges in maintaining normal operations amid high case volumes.

In terms of clinical presentation, Omicron infections typically result in milder symptoms, such as cough, sore throat, nasal congestion, and fatigue, with fewer cases progressing to severe respiratory distress compared to prior variants. However, the large

number of infections has still resulted in increased hospitalizations and, consequently, fatalities due to the strain on healthcare capacity. Research from South Africa and other countries suggests that hospitalization rates during Omicron waves were lower than those observed during Beta and Delta waves. Nevertheless, Omicron's impact varies widely depending on factors such as vaccination status, previous infection history, and individual health conditions (Fan et al., 2022).

The emergence of Omicron has significantly reshaped the COVID-19 pandemic landscape. Its high transmissibility and immune evasion capabilities have posed ongoing challenges for vaccination strategies and other public health interventions. The reduced effectiveness of two-dose vaccine regimens against Omicron has prompted many health agencies to recommend booster doses, which have shown some efficacy in enhancing immunity against this variant and reducing severe outcomes. Additionally, Omicron's rapid spread and ability to reinfect have sparked further research into variant-specific vaccines and next-generation immunization strategies. The ongoing evolution of the Omicron variant has led to numerous subvariants, such as BA.4, BA.5, and more recently, XBB. These subvariants often carry additional mutations that may further enhance transmissibility or alter immune evasion capabilities. Continuous monitoring of these subvariants is essential to track any shifts in infectivity, immune escape, or disease severity. The Omicron variant underscores the importance of genomic surveillance, adaptive public health policies, and robust healthcare systems as the virus continues to evolve (Arabi et al., 2023).

2.1.6 Transmission

The SARS-CoV-2 virus, which caused the COVID-19 pandemic, is believed to have originated in bats, with an unidentified intermediary host facilitating its transmission to humans. Bats are regarded as the likely reservoir, similar to patterns seen with other coronaviruses. Although the exact pathway remains partially speculative, the initial outbreak of COVID-19 was traced to the Huanan seafood market in Wuhan, China. However, subsequent cases with no market exposure revealed that human-to-human transmission was already occurring early in the outbreak (Mehraeen, 2024). SARS-CoV-2 spreads primarily via respiratory droplets released when infected individuals cough, sneeze, or speak. While droplets settle quickly and can contaminate surfaces, aerosols—smaller particles capable of remaining airborne—can travel greater distances, heightening transmission risk through both direct exposure to infected individuals and contact with contaminated surfaces nearby (Karia et al., 2020).

Infected individuals typically carry a high viral load in their upper respiratory tracts and may continue to shed the virus in saliva and faeces for up to two weeks post-recovery, though the role of faecal shedding in transmission remains inconclusive. While the possibility of faecal-oral transmission exists, isolating the virus from stool samples has proven challenging (Li et al., 2020). Multiple factors influence the risk of SARS-CoV-2 spread, including frequency and proximity of contacts, environmental conditions, host infectiousness, and socioeconomic factors. The majority of transmissions occur in close-contact settings, especially within households and through social gatherings. Household secondary attack rates vary widely, from 4% to as high as 35%, with activities such as sharing a room, dining together, or engaging in group activities with an infected person presenting elevated risk (Li et al., 2020).

COVID-19 primarily spreads via droplet transmission, generally occurring within proximity—often under 1 meter and rarely extending beyond 2 meters—from an infected individual who is coughing, sneezing, or even speaking. This transmission risk is heightened at mucosal entry points, such as the respiratory tract, which is highly receptive due to the abundance of ACE2 receptors in the human respiratory epithelium, notably in the oropharynx and upper airways. The ocular and gastrointestinal tracts also offer potential entry points for the virus, increasing the scope of susceptibility. Larger respiratory droplets tend to settle near the source, while finer droplets and aerosols remain airborne for longer durations, contributing significantly to community transmission. In response, health authorities globally and within Malaysia advocate for preventive measures—mask-wearing, rigorous hand hygiene, and social distancing—especially in crowded or confined areas, aiming to curb the spread effectively (World Health Organization, 2024).

While droplet transmission dominates, airborne transmission remains a notable risk in specific high-exposure settings where aerosol-generating procedures are common, such as endotracheal intubation, bronchoscopy, open suctioning, and CPR. Aerosols, which contain viral particles within droplet nuclei smaller than 5 micrometres, can persist in the air for prolonged periods and disperse over distances greater than 1 meter. Environmental factors like ventilation, temperature, and humidity in enclosed spaces significantly influence airborne transmission (Zhao et al., 2022). Poorly ventilated indoor environments—including public transit, classrooms, office spaces, and dining venues—are particularly vulnerable.

According to the latest WHO data, COVID-19 may also spread through contact with fomites, as contaminated droplets can survive on surfaces for hours to days, depending on the material and environmental conditions. On surfaces like metal, glass, or plastic, SARS-CoV-2 may remain infectious for up to 9 days, with extended viability under cold, dry conditions. Hence, surface disinfection and regular hand hygiene are indispensable in mitigating SARS-CoV-2 transmission. Moreover, asymptomatic carriers, though often overlooked, contribute to the virus's spread. Transmission may occur through seemingly innocuous actions such as touching the face, spitting, or speaking, emphasizing the critical need for universal preventive practices. The commitment to these measures has been instrumental in controlling outbreaks, especially in public spaces, where viral load and transmission risk are highest (Galbadage et al., 2020).

2.1.7 Life Cycle

The life cycle of SARS-CoV-2 within a host cell is a complex, multi-stage process involving several precise and interdependent phases: viral entry, translation of viral replication components, genome replication, synthesis of structural proteins, virion assembly, and ultimately, viral release (Guo et al., 2020). This sophisticated process begins with an intricate host-pathogen interaction. The virus's spike protein binds with high affinity to the angiotensin-converting enzyme-2 (ACE2) receptors predominantly found on the surface of bronchial and intestinal epithelial cells. This binding facilitates viral entry and initiates the downregulation of ACE2 expression, a key event leading to severe respiratory dysfunction in infected individuals.

Once internalized through endocytosis, the S1 spike protein undergoes proteolytic cleavage via host cellular enzymes, including cathepsin, transmembrane protease serine 2 (TMPRSS2), and trypsin. This cleavage exposes the S2 subunit (fusion peptide), enabling the viral envelope to fuse with the endosomal membrane and allowing the viral capsid to release into the cytoplasm. Cells expressing both ACE2 and TMPRSS2 are especially vulnerable to viral entry.

Following successful entry, the viral positive-sense single-stranded RNA (ssRNA) utilizes the host cell's ribosomes to synthesize viral RNA-dependent RNA polymerase, critical for replication. The viral RNA is then replicated into a double-stranded RNA (dsRNA), allowing further synthesis of viral genomes. The nucleocapsid (N) protein is produced in the cytoplasm, while the spike (S), membrane (M), and envelope (E) proteins are synthesized on the rough endoplasmic reticulum (RER) and modified at the subgenomic level. These proteins undergo meticulous post-translational processing to achieve structural functionality.

Finally, all viral components are assembled in the Golgi apparatus to form mature virions encased in lipid envelopes. These fully developed viral particles are released from the infected cell through exocytosis, making them primed to infect additional host cells. This life cycle epitomizes the viral efficiency and adaptability that have contributed to the global spread and persistence of SARS-CoV-2, which has now led to millions of cases and fatalities worldwide (Jackson et al., 2021).